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Journal of the Alabama Academy of Science, Vol. 74, No. 1, January 2003.

EFFECT OF 4-ETHYL-4-AZA-5α-CHOLESTANE(ND-497) ON CARBOHYDRATE METABOLISM OF *STAPHYLOCOCCUS AUREUS*

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ABSTRACT

The steadily increasing number of antibiotic resistant strains of infectious microorganisms is frightening. There is special concern over *Staphlococcus aureus*, the principal cause of hospital infections, boils, cellulitis, toxic shock syndrome and death from infection. Novel potent antimicrobial azasteroids, active against bacteria, molds and yeasts, have been discovered in our steroid research. We wish to describe recent studies on ND-497 (4-ethyl-4-aza-5α-cholestane) against a penicillin-resistant strain of Staph. It was found to be a more powerful inhibitor of this resistant strain than erythromycin, streptomycin, polymyxin B, neomycin or tetracycline.

These azasteroids inhibit growth of gram-positive bacteria and fungi at 1.0 μg/ml or less. It appeared at first that activity resulted from damage to cellular membranes and a loss of essential constituents. We have now discovered that the azasteroids are specific in their action and toxic at concentrations that do not cause leakage. They disrupt energy production at low concentrations. Future research will seek to identify affected enzymes and proteins.

INTRODUCTION

Increased personal hygiene, safe water supplies, vaccines, sulfa drugs and antibiotics had increased life expectancy from 47 to almost 80 years in a century. Now, emerging

new infectious disease problems, the development of pathogens resistant to anti-infective drugs and the spectre of bio-terrorism are frightening. During the past 25 years few new antimicrobial drugs were introduced and most of these were "cousins" of drugs in use and prime candidates for microbial resistance. Adverse side effects are also being observed with increasing frequency prohibiting the use of certain drugs on some patients. Enserink (2003) describes a new strain of resistant staph, producing painful boils on many parts of the body, pain and death, which is epidemic in Aids communities and prisons. He expresses the fear that this strain will become established in our hospitals. Shnoyerson and Plotkin (2002) shared a frightening scenario in their book, "the Deadly Rise of Drug Resistant Bacteria." The possibility of being exposed to dangerous and difficult to treat infectious diseases has become a reality.

Since the mid-1950's, Doorenbos's laboratory has been developing procedures for synthesizing target heterocyclic steroids as potential medicinal substances. Novel androgen, progesterone, and aldosterone inhibitors, as well as anti-inflammatory, antibacterial, antifungal, neuromuscular blocking, and birth control products have been discovered in these studies.

Azasteroids, steroids in which one or more of the skeletal carbon atoms is replaced by nitrogen, proved to be the most interesting of the heterocyclic steroids. Azasteroids do not occur in nature. They must be synthesized. Beginning with position 4, methods for introducing a nitrogen atom at almost every position were developed. The first designed 4-aza-steroids were prepared in our laboratory. It was in the late 1950's. 4-Aza-5-androsten-17β-ol-3-one and several derivatives were prepared It was anticipated that they would be competitive antagonists of testosterone rendering them useful in delaying the development of enlarged prostate glands and prostate cancer as well as treating these conditions. These studies were supported by the National Cancer Institute. Finasteride and Proscar, first marketed around 1980, are two analogues of these 4-azasteroids in use today.

An antimicrobial screening program, inaugurated specifically for our heterocyclic steroids in 1960, identified several very active compounds (Doorenbos 1962 1964, Shay 1962, Smith 1963 1964, Varrichio 1966). Most were azasteroids. The first announcement of antimicrobial azasteroids reported the inhibitory activity of five steroids on resting and growing cells of *Sarcina lutea* and *Saccharomyces cerevisiae* at concentrations of 1 to 100 μg/ml. Additional studies with sixteen nitrogen-containing steroids (Smith 1964) revealed that four 4-azacholesanes inhibited a large number of gram-positive bacteria, yeasts and molds at concentrations often less than 1 μg/ml. Growth of *Gaffkya tetragena,* the most sensitive microorganism, was inhibited at 0.02 μg/ml. *Brucella abortus* was the only observed sensitive gram-negative bacterium in these initial studies.

An investigation of effects of five of these steroids on *Streptococcus pyogenes* (Smith 1963) demonstrated a similarity with cationic quaternary ammonium salts. Each are complexed by serum proteins, are hemolytic, inhibited by lecithin and fatty acids, and lower surface tension. However, the many observed differences made it clear that there was much more to mechanisms of action.

Many variations in structural features of azasteroids markedly affected activity suggesting interactions with enzymes, proteins or other receptors are significant in their mechanisms of action. For example, azaandrostanes and azapregnanes are inactive, unlike the azacholestanes (Smith 1963 1964)). Azacholestane derivatives with a 5β-configuration

are inactive (Scott 1966). A 3β-alkyl group enhances activity and a 6β-alkyl group destroys activity (Doorenbos 1965).

Two mechanisms affecting cell permeability were revealed in studies with the yeast, *Saccharomyces cerevisiae.* Sublethal concentrations of azasteroid blocked uptake of glucose and alanine by viable resting cells as did uranyl nitrate (Smith 1964). Uranyl nitrate is known to be a specific inhibitor of glucose transport. Yeast cells, pretreated with uranyl nitrate, were protected from the lethal action of these steroids. Thus it appears that these substances competitively bind at the same site which is involved in nutrient uptake.

Nutrient uptake inhibition would be growth limiting but would not explain the primary lethal action of the azasteroid. It was postulated that azasteroid inhibition of cell growth was due to effects on essential enzymes or other proteins inhibiting essential functions such as energy production. Examination of the effects of ND-497 on *S. lutea* revealed that it inhibited dehlydrogenase of whole cells or lysates regardless of whether the systems were using endogenous, glucose or succinate metabolism (Smith 1965).

METHODS

The azasteroid selected for these studies was 4-ethyl-4-aza-5α-cholestane (ND-497), Figure l, (Doorenbos 1962), prepared synthetically from cholesterol.

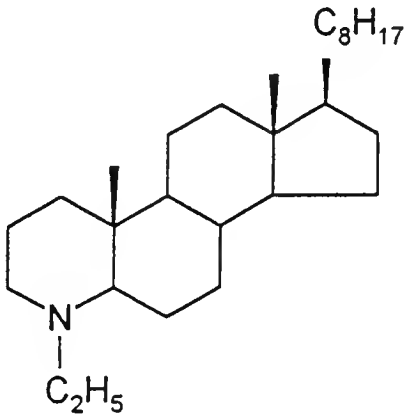

Figure 1. Cultures: Microorganisms used in studies on ND-497, obtained from the Dept. of Biology, University of Mississippi, included *Sarcina lutea, Staphylococcus aureus, Mycobacterium smegmatis, Pseudomonas seruginosa, Serratia marcescens, Trichophyton, mentagrophytes, Candida albicans,* and *Saccharomyces cerevisiae.*

A penicillin resistant strain of *S. aureus* K257 culture was selected for the respiration studies. Stock cultures were maintained on nutrient agar slopes in the cold and subcultured bimonthly.

Media: Media were obtained from Baltimore Biological Laboratories and sterilized by autoclaving, Bacteria were maintained on slants of brain heart infusion agar and yeasts and molds on Sabouraud dextrose agar.

Staphlococcus aureus

Medium used for *S. aureus* contained: 20 g. pancreatic digest of casein, 110 μg biotin, 50 μg nicotinic acid, 50 μg thiamine hydrochloride in 1.0 l. distilled water adjusted to pH 7.6 with sodium hydroxide and autoclaved. Fresh egg white was added to achieve 1.0% v/v. Cultures were incubated 12 hours at 37°C. Cells were washed with a phosphate buffer (pH 7) prior to respiration experiments.

Substrates and Test Compounds: 0.05 M solutions of the following were sterilized by filtration: glucose, sucrose, D-glucose-6-phosphate disodium salt, D-glucose-1-phosphate dipotassium salt, sodium citrate, cis-aconitic acid, and α-ketoglutaric acid. The following test compounds wee prepared at 120 μg/ml and 60 μg/ml: ND-197, uranyl nitrate, polymyxin B sulfate, streptomycin sulfate, neomycin sulfate, eythromycin and tetracycline hydrochloride.

Antimicrobial Activities of ND-497: Minimum inhibitory concentrations against the various microorganisms were determined, repeating each four times, by the two-fold serial dilution method in Eugon broth.

Respiration Studies: Duplicate measurements utilizing standard Warburg manometric techniques were used for measuring oxygen uptake.. Cells were harvested by centrifugation and washed twice with phosphate buffer prior to use. Oxygen uptake was measured in microliters at 15 min. intervals at 37°C.

RESULTS

This penicillin resistant strain of *S. aureus* exhibited four times the sensitivity to ND-497 of the other seven microorganism species assayed. It was sensitive at 10 μg/ml, the lowest concentration used in these studies. The rate of respiration of *S. aureus* was highest for cells metabolizing glucose, intermediate for fructose and lowest for sucrose. ND-497 substantially inhibited the metabolism of each of these sugars at concentrations well below that producing lysis. The lowest concentration used in these studies was 10 μ ug/ml. Similarly, ND-497 inhibited the metabolism of pyruvate, glucose-1-phosphate, glucose-6-phosphate, Krebs cycle and pentose cycle intermediates. This is illustrated by averages of duplicate respiration measurements of microliters of oxygen uptake per flask in 105 minutes (Table l).

Comparisons of inhibtion of glucose metabolism were made between ND497 and several antimicrobial agents known to inhibit carbohydrate metabolism.. It was discovered that not only was ND-497 the most potent of these substances, but also had the greatest effect in reducing glucose metabolism at each of several concentrations over the 105 minutes of each experiment. No evidence of the presence of resistant cells was observed in a series of gradient plate studies. Combinations of each of these antimicrobial agents and ND-497 were also evaluated. Tetracycline hydrochloride, erythromycin and uranyl nitrate each inhibited the actions of ND-497. Synergistic responses were observed when ND-497 was used in combination with neomycin sulfate, streptomycin sulfate or polymyxin B sulfate (Table 2).

DISCUSSION

ND-497 is one of over thirty novel potent antimicrobial substances discovered in Doorenbos's research. They are heterocyclic derivatives of natural steroids active against gram-positive bacteria, molds and yeasts. The goal of this study was to learn more about possible mechanisms of action.

Earlier studies had demonstrated that these substances damage the microbial cell membranes causing leakage of cellular constituents.. There was evidence of antimicrobial activity at concentrations which did not induce cell leakage. This observation is confirmed in this study which demonstrated inhibition of metabolism

TABLE 1

Warburg Respiration Measurements (Average of two measurements)

Substrates	Oxygen Uptake (μL105 min.)
Glucose	190
Fructose	175
Sucrose	155
Glucose + 20 μg ND-497	135
Fructose + 20 μg ND-497	130
Sucrose + 20 μg ND-497	105
Glucose-6-phosphate	47
Glucose-6-phosphate + 20 μg ND-497	18
Glucose-1-phosphate	39
Glucose-1-phosphate + 20 μg ND-497	35
Citrate	75
Citrate + 20 μg ND-497	67
Pyruvate	73
Pyruvate + 20 μ ND-497	64
cis-Aconitate	48
cis-Aconitate + 20 μg ND-497	18
α-Ketoglutarate	33
α-Ketoglutarate + 20 μg ND-497	28

TABLE 2

Warburg Respiration Measurements (Average of Two Measurements)

Antimicrobial Substrate	Oxygen Uptake (μL 105 min.)
20 μg. tetracycline hydrochloride	89
10 μg tetracycline hydrochloride + 10 μg ND-497	71
10 μg ND-497	66
20 μg ND-497	64
20 μg erythromycin	91
10 μg erythromycin + 10 μg ND-497	80
20 μg streptomycin sulfate	70
10 μg streptomycin sulfate + 10 μg ND-497	62
20 μg polymyxin B sulfate	72
10 μg polymyxin B sulfate + 10 μg ND-497	62
20 μg neomycin sulfate	87
10 μg neomycin sulfate + 10 μg ND-497	62

of glucose, fructose, sucrose, pyruvate, glucose-1-phosphate and glucose-6-phosphae without inducing cell leakage. These results demonstrate effects upon enzymes involved in oxidation/reduction

The discovery of antagonism of the effects of ND-497 by tetracycline, erythromycin and uranyl nitrate suggests that similar enzymes or proteins are involved. The synergistic effects of neomycin, streptomycin and polymyxin B indicates that they have different mechanisms of action and affect different enzymes and proteins. It is noteworthy that ND-497 was a more potent antimicrobial agent and inhibitor of carbohydrate metabolism of this strain of penicillin resistant *Staphlococcus aureus* than any of the antibiotics included in this study.

Research to identify affected proteins, enzymes, and other receptor sites, structural requirements, active sites and mechanisms of action are in progress.

ACKNOWLEDGEMENTS

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HYPOCHOLESTEROLEMIC 3β-BENZYL-4-METHYL-4-AZA-5α-CHOLESTANE METHIODIDE (ND-354)

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ABSTRACT

Since the mid-1950's, my laboratory has been developing procedures for and synthesizing target heterocyclic steroids as potential medicinal substances. Novel androgen, progesterone, and aldosterone inhibitors, as well as anti-inflammatory, antibacterial, antifungal, neuromuscular blocking, and birth control products have been discovered in these studies. Among these heterocyclic steroids is 3β-benzyl-4-methyl-4-aza-5α-cholestane methiodide (ND-354), described in 1962 as a novel antimicrobial steroid. This paper focuses on the hypocholesterolemic properties.of ND-502.

INTRODUCTION

Heterocyclic steroids are steroid derivatives in which one or more of the skeletal carbon atoms have been replaced by a nitrogen, oxygen, sulfur or other type of atom. This definition has been extended to include steroids with a heterocyclic ring fused or attached to the steroid skeleton. Having discovered that several of our azasteroids (Doorenbos 1962, Doorenbos and Wu 1965}, and published aminosteroids (Counsel, Ranney and Cook 1962, Counsel, Klimstra, Nysted and Ranney 1965) inhibited steroid biosynthesis, we sought to synthesize clinically useful derivatives.

EXPERIMENTAL

Our more active hypocholesterolemic steroids were ring A azacholestanes. These included 2-aza-5-α-cholestane and 3-aza-5α-cholestane (Doorenbos and Havranek 1965, Doorenbos, Vaidya and Havranek 1967), 4-aza-5α-cholestane and 4-aza-5β-cholestane (Scott 1966). Also investigated were 3β-alkyl and 3β-aryl (Doorenbos and Kerridge 1965) and 6β-methyl (Doorenbos and Bossle 1965) substituted 4-aza-5α-cholestanes plus N-methyl and methiodide quaternary salt derivatives of each of these azasteroids. With the exception of the 5β-cholestane derivatives, each compound exhibited hypocholesterolemic activity. The 4-aza-5α-cholestane methiodides were the most active. One of these derivatives, 3β-benzyl-4-

methyl-4-aza-5α-cholestane methiodide (ND-354), described in 1962 as an antimicrobial steroid (Doorenbos 1962), is the subject of this report.

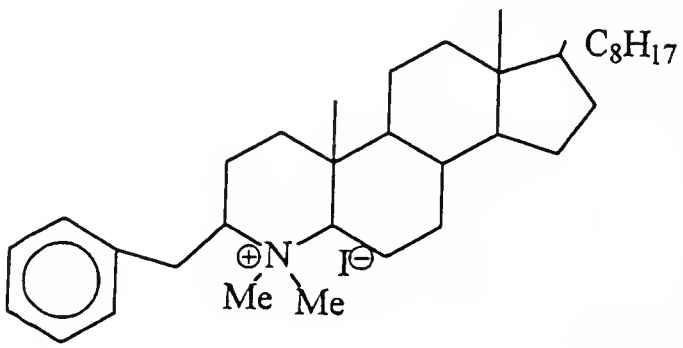

Figure 1. 3β-Benzyl-4-methyl-4-aza-5α-cholestane ethiodide(ND-354)

Special interest developed in these more active 4,4-disubstituted azacholestanes, reasoning that such derivatives might be effective by one or more of three mechanisms: They might (1) inhibit cholesterol absorption from the GI tract by forming poorly absorbed cholesterol complexes, (2) complex bile acids making them unavailable to facilitate cholesterol absorption and thus stimulating increased liver conversion of cholesterol to bile acids or (3) inhibit cholesterol biosynthesis, hopefully at the lanosterol step. It was postulated that inhibition of lanosterol biosynthesis from squalene could reduce cholesterol levels without a concomitant rise in the levels of the sterol intermediates, lanosterol, zymosterol and desmosterol. It was recognized also that cellular absorption of ND-354 would probably be limited since it was a aquaternary salt.

Noteworthy are (a) the unusual structural features of ND354, (b) the use of a Grignard reaction of a lactam in its synthesis; (c) cholesterol inhibition by action at the lanosterol stage without a build up of sterol precursors and (d) absence of adverse side effects in animal or human studies.

MATERIALS AND METHODS

3β-Benzyl-4-methyl-4-aza-5α-cholestane methiodide (ND-354) was prepared as described (Doorenbos and Kerridge 1966) in a seven step synthesis from cholesterol. Cholesterol was oxidized to 4-cholesten-3-one by Oppenauer oxidation. Subsequent treatment with ozone yielded 3,5-seco-4-nor-5-cholestanon-3-oic acid, which was converted to 4-methyl-4-aza-5-cholesten-3-one, an enamine lactam, by reaction under heat and pressure with methylamine. Grignard reaction with benzylmagnesium iodide provided 3-benzyl-4-methyl-

4-aza-2,5-cholestadiene which was hydrogenated over a platinum catalyst to 3β-benzyl-4-methyl-5α-cholestane. Subsequent treatment with methyl iodide yielded ND-354.

ND-354 was obtained as white crystalline needles. It readily formed a hydrate but presented no problems in formulating research preparations or effective standard liquid, tablet or capsule dosage forms. Preclinical studies were conducted with mice, rats and dogs followed by, after FDA approval, preliminary clinical studies.

RESULTS

Cholesterol biosynthesis in rat liver homogenates was curtailed up to 90% by 0.001 M concentrations of ND-354. In vivo rat studies with labeled acetate indicated cholesterol synthesis was blocked between acetate and mevalonate.

Inhibition of lipid absorption from the gut was observed upon oral administration and attributed to the formation of complexes with bile acids. Oral doses as low as 5 mg/kg b.i.d. in mice reduced liver and plasma cholesterol. Oral doses of 10 mg/kg b.i.d. in dogs significantly lowered plasma cholesterol and phospholipid levels. No other pharmacology was noted. No cardiovascular, significant endocrine, CNS or toxicity effects were observed upon oral or subcutaneous administration. This included oral doses as high as 1000 mg/kg in dogs and 2000 mg/kg in rats. administered for two weeks. Oral doses of 1.0 g/day in patients reduced cholesterol levels. Oral doses as high as 2.0 g/day were administered for four weeks without any adverse reactions.

DISCUSSION

ND-354 is unlike natural steroids in that it contains a nitrogen rather than carbon at position 4 and an organic rather than an oxygen functional group at position 3. The overall 3-dimensional shape, however, is similar to desmosterol except for the presence of the large benzyl group at position 3. It appears evident that this functional group does not interfere with adsorption to key receptors. Similarly the presence of a positive charge at position 4 is not inhibiting adsorption. It is possible that the positive charge at position 4 and or the organic group increase the strength of adsorption to key receptors. Comparative affinity adsorption studies are planned.

The absence of toxicity or adverse effects upon oral or parenteral administration were encouraging and similar to observations made in other studies of related azasteroids.

Those conducting the animal and clinical studies on NID-354 described it as an effective plasma cholesterol lowering agent. Failure of negotiations with N.I.H., which supported the synthetic studies, to gain approval of a limited exclusive license to market ND-354 contributed to the decision to close out the clinical studies.

ACKNOWLEDGEMENTS

We wish to acknowledge with thanks the support of research grant CA-04132 from the National Cancer Institute and gifts of steroid intermediates from the Cancer Chemotherapy National Service Center, National Institutes of Health. We also wish to thank Smith Kline & French Laboratories for sharing in vivo and clinical information on samples sent to them for evaluation.

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A PRELIMINARY ASSESSMENT OF THE EFFECTIVENESS OF THE ALABAMA ENTERPRISE ZONE PROGRAM, 1986-2001

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ABSTRACT

This study explores the effectiveness of the design, implementation, and economic impact of the state enterprise zone (EZ) program in Alabama during the 1986-2001 period by using data provided by Alabama Department of Economic and Community Affairs (ADECA), surveys, and interviews with zone coordinators and participating businesses. Due to a low survey response rate, meaningful findings were difficult to achieve. However the study suggests that poor record keeping practices by state and local zone coordinators and participating businesses, a lack of professionalism by state and local officials, a lack of communication among parties involved in the zone program, and a lack of resources (poor staffing, insufficient financial and technical assistance to participating businesses) has significantly hindered effective operation of the EZ program in Alabama.

INTRODUCTION

The pursuit of effective economic development and revitalization strategies has continued in the face of urban decline and deterioration in America in the past. In many states, one innovation among local economic development and revitalization initiatives has been the use of enterprise zones (EZ) since the 1980s. The use of enterprise zones as a tool to stimulate private investment and jobs in economically distressed areas by providing tax incentives and regulatory relief began in the late 1970s in England. Actually, the British enterprise zone derived from the economic success of enterprise zone-type policy precedents established in Hong Kong and in Taiwan (Butler, 1991; Liebschutz, 1995).

In the United States, the viability of the enterprise zone concept has been debated on the federal level since it initially appeared in the bipartisan Urban Jobs and Enterprise Zones bill introduced in the U.S. House in 1980 by Congressman Jack Kemp (R-N.Y) and Robert Garcia (D-N.Y). Former President Reagan promoted the enterprise zone idea in his 1980 campaign and again in 1984, as did President Bush in his State of the Union address in 1990. While the enterprise zone concept has had executive branch support for a decade and the number of

enterprise zone concept has had executive branch support for a decade and the number of congressional supporters of zone legislation has continued to grow, the only federal enterprise zone legislation enacted before 1996 was Title VII (Enterprise Zone Development) of the Housing and Community Development Act of 1987. However, Title VII did not provide any tax benefits but focused on the coordination of existing federal program and the reduction of government regulations. The idea of the federal enterprise zone program was in part realized in the Empowerment Zones and Enterprise Communities (EZEC) Act that was passed by Congress in May 1993 under the Clinton Administration (Rubin, 1994; Liebschutz, 1995). The Act provides federal tax incentives to participating businesses and substantial direct federal government expenditures ("government investment") at targeted zone communities.

While the idea of enterprise zone legislation struggled at the national level in the 1980s and early 1990s, some states picked up the theme and proceeded to create enterprise zones of their own during this period. Connecticut, New Jersey, Maryland, and Illinois were among the most active states in establishing enterprise zones. By 1995, 34 states and the District of Columbia had created over 3,000 enterprise zones (Wilder and Rubin, 1996).

Following the lead of other states, the Alabama Legislature passed legislation in 1987 to set up 25 state enterprise zones in anticipation of the development of federal enterprise zones (Alabama Department of Economic and Community Affairs, 2000). Although Alabama's EZs have been around for more than a decade, no systematic study has yet examined their design, implementation, and economic impact (such as employment growth and capital investment). Preliminary as it is, this study attempts to provide such an analysis using data provided by Alabama Department of Economic and Community Affairs (ADECA) and secured from a mail survey sent out to all EZ coordinators and EZ businesses in the state.

Following this introduction, section two of this article reviews the literature related to the enterprise zone programs, focusing on the impact of zone programs on economic growth and capital investment. Section three describes the methodology used in the study, and section four discusses research findings. Section five discusses problems associated with the EZ program in Alabama and suggests ways to improve it by comparing it with EZ programs in California. The final section of this article identifies the study's limitations and indicates avenues for future research.

LITERATURE REVIEW

More than 40 studies have examined the effects of enterprise zone programs and other issues related to them (Wilder and Rubin, 1996; Elling and Sheldon, 1991). Most of the research on EZs centers on two key questions: (1) Do enterprise zone incentive programs generate new employment and investment in declining urban areas? (2) What are the direct and indirect costs and burdens to enterprise zones (cost-effectiveness of EZ program)?

Empirical analysis on the impact of zone programs is somewhat limited due to two basic constraints: the lack of reliable quantitative data to evaluate zone performance and the difficulty of isolating the zone designation and incentives from those of other factors (Wilder and Rubin, 1996; Elling and Sheldon, 1991; Dowall, 1996). Because of such methodological problems, researchers have employed a wide range of research methods, including case studies, multivariate statistical analyses, and surveys of zone operators and firms (Boarnet, 2001; Wilder and Rubin, 1996). Some studies have focused on assessments of a single zone or area (Lambert and Coomes, 2001; Rubin and Wilder, 1989) while a few have examined a number of zones within a state (Dowall, 1996; Rubin, 1991; Papke, 1991) or multi-state region (Elling and

Sheldon, 1991; US Department of Housing and Urban Development, 1986; Erickson et al., 1989). Regarding data collection, researchers have drawn primary data from businesses and zone administrators as well as secondary data on employment and firms from local, state, and federal government sources. Considering the variety of research methods and data sources, it is no surprise that the results of these studies are diverse, contradictory, and difficult to generalize.

In measuring zone effects, researchers have utilized one of the three approaches: (1) comparing economic performance of zones with their surrounding areas before and after zone designation using secondary data; (2) comparing levels of economic activity in control communities against zone communities using secondary data on employment, establishments, and output; (3) gauging the effects of zone programs and incentives on business location and expansion decisions by directly surveying businesses and zone administrators (Dowall, 1996; Boarnet, 2001; Wilder and Rubin, 1996).

The before-and-after studies of zones and adjacent areas found little or no difference between the levels of zone and adjacent area economic activities after the introduction of the enterprise zone program to the community (Boarnet, 2001; Wilder and Rubin, 1996). Studies comparing zone and control communities found that both employment and economic activity in the zones were greater than in control areas, although the differences were not statistically different (Dowall, 1996; Wilder and Rubin, 1996). In any case, both of the approaches relying on indirect assessments of the economic effects of zone activities were inconclusive. The direct surveys of businesses and program administrators in such zones, however, tended to produce positive perceptions of the impact of zones. This positive image of zones may be in part due to zone officials' natural tendency to attribute any job growth within the zone exclusively to the enterprise zone's influence (Boarnet, 2001; Wilder and Rubin, 1996).

Each method used has its strengths and weaknesses. Better research would be possible if researchers could combine several methods to measure the effects of enterprise zone programs and incentives. For example, an empirical analysis could be made of employment and establishment trends that covered pre- and post- zone designation. In addition, such trends can be compared with data from surveys of businesses and administrators to determine whether observed changes in employment and establishments could be attributed to zone activities (Dowall, 1996; Wilder and Rubin, 1996).

Table 1 summarizes the findings over 40 studies accomplished on the impact of EZ programs. These works are selected because they either provide major findings or address important methodological issues. Early studies were mostly descriptive case studies which analyzed a single zone or several zones in a state or multi-state area. These studies used survey and interview data of state and local zone coordinators and business establishments (U.S. Department of Housing and Development, 1986; California Office of the General Auditor, 1988; U.S. General Accounting Office, 1988). Findings on the impact of zones on job creation and investment of these descriptive case studies dramatically varied among states and even different zones within a single state. For example, the HUD study (1986) that looked at enterprise zones in nine states showed that although some zones had a dramatic and steady growth in investment, others had experienced an initial growth followed by a noticeable slowdown. Still other zones remained stagnant and continued to decline economically.

The study acknowledged the difficulty of obtaining reliable data on zone effects and cautioned that the observed outcomes for jobs and investment could not be totally attributed to the enterprise zone programs. This inconclusiveness was due to the fact that the study was based on interviews with zone officials, who are potentially biased by a natural tendency to attribute

any job growth within the zone exclusively to the enterprise zone's influence. Moreover, it should be noted that state and local officials tend to ignore job losses and focus on gross rather than net growth over time. In addition, local officials tend to provide somewhat inflated estimates of job retention, a condition which is difficult to measure (Boarnet, 2001; Wilder and Rubin, 1996). Thus, the issue of data bias and/or reliability should be raised regarding studies that rely heavily on interviews and perceptions rather than more rigorous measures.

Table 1. Summary of Selected Studies on Enterprise Zones

Author(s) and year published	Geographic area (study time period)	Measure	Methodology	Findings
Lambert and Coomes (2001)	Louisville, KY (1982-96)	jobs, capital investment	shift-share analysis	negative
Dowall et al. (1994)	CA (1986-90)	employment growth/ business investment	shift-share analysis	modest/mixed
Elling and Sheldon (1991)	IL, IN, KY, OH (start-ups to 1988)	ability of zones to attract firms	multiple regression	modest
Rubin (1991)	NJ (1987-92)	tax revenue from investment	input-output analysis	positive
Papke (1991)	IN (1987)	jobs	cost/jobs	positive
Rubin and Wilder (1989)	Evansville, IN (1983-86)	comparative advantage of the zone	shift-share analysis	positive
Erickson et al. (1989)	357 zones in 17 states (start-up to 1986)	jobs/investment	correlation/regression	positive
U.S. General Accounting Office (1988)	MD (1980-87)	employment levels	interrupted time series	negative
California Office of the Auditor General (1988)	CA (1986-87)	employment growth	descriptive statistics	positive
U.S. Department of Housing and Urban Development (1986)	Bridgeport, CT	jobs	local official's assessments	positive
	Chicago, IL	unemployment	------	modest
	Dayton, OH	unemployment rate	------	little change
	Louisville, KY	jobs	------	positive for small firms
	Macon, MO	investment, jobs	local official's assessments	positive
	St. Louis, MO	jobs to residents	------	negative
	Michigan City, IN	potential success	------	modest
	Tampa, FL	firm decision to invest	------	modest
	Thief River Falls, MN	location decisions	interview with firm representatives	modest
	York, PA (program start-up dates to 1985)	location decisions	local official's assessment	negative

------ represents same as the above.

In order to reduce data bias and reliability problems, the second wave of research (analytic research) attempted to find causal relations by addressing the following research questions (Boarnet, 2001; Wilder and Rubin, 1996): (1) what factors explain the variation in zone effects? (2) can observed changes in economic activity be directly attributed to enterprise zones? In analyzing causal relationship, researchers employed research methods beyond survey and interview methods, which included multiple regression, shift-share analysis, and input-output analysis (Erickson et al., 1989; Elling and Sheldon, 1991; Rubin, 1991; Dowall et al., 1994; Lambert and Coomes, 1996; Wilder and Rubin, 1988).

Research by Rubin and Wilder (1989) determined the extent to which new job development was attributable to the enterprise zone programs in the Evansville zone in Indiana by employing shift-share analysis. The study showed that between 1983 and 1986, a net increase of 1,878 jobs occurred in the Evansville zone. Of this total, 325 jobs resulted from general economic growth in the metro area, and 123 jobs from the zone's industrial composition. Only the remaining 1,430 jobs were attributed to the comparative advantage of the enterprise zone. In their previous study (Wilder and Rubin, 1988) of the same zone area, they concluded that the relative success of the Evansville zone was due to the economic viability of the zone area, a high concentration of industrial land use, the autonomy of the zone's administration from the government, the credibility and competency of the zone manager, and the community organization role played by the zone association.

Research by Erickson et al. (1989) provides additional insight on the effect of zone programs. Analyzing HUD's cross-sectional data on 357 enterprise zones in 17 states, and 2,014 business establishments, the authors examined the relationship between the structural characteristics of enterprise zone programs (e.g., number/type of incentives, number/size of zones) and the observed changes in employment and investment. The regression model demonstrated a strong positive relationship between the number of business incentives and job growth, and a positive relationship between the number of incentives provided to zone businesses and the amount of investment by those businesses. In order to complement their study, the researchers also interviewed the coordinators of 21 high performance zones. From this survey, the authors identified four common explanations for zone success. First, the zone encompassed an area that was still economically viable. Second, the EZ designation served as a catalyst and/or stabilizer, but was not the sole determining factor in revitalization. Third, the number and variety of zone incentives added to a program's effectiveness. Fourth, strong local support from the private and public sectors increased the success of the program.

To summarize, the variability in job growth and investment found among different state programs, as well as between zones within the same states, indicates the limits of the effect of enterprise zone programs. It suggests that the designation of state enterprise zones does not have magical qualities that can overcome all physical, social, and economic barriers to revitalization. It seems that the myriad of social and physical problems plaguing many urban neighborhoods (e.g., decaying infrastructure, high crime rates, inadequate school systems) are not responsive to targeted development incentives as the critics of the zones argue. Enterprise zones seem to stimulate new employment and investment in certain areas and much attention must be focused on program features in order to increase the probability of desired outcomes (Wilder and Rubin, 1996). Previous literature suggests that the revitalization of declining areas requires a comprehensive, coordinated, and planned set of strategies aimed at increasing economic opportunities for residents as well as for business owners.

METHODOLOGY

In order to gather exploratory and descriptive information on how the 27 Alabama Enterprise Zones are designed and implemented, as well as how they have performed, we developed a mail survey for both program participants (EZ businesses) and zone coordinators. The surveys contained questions regarding several characteristics of the program including; (1) the characteristics of zone design (including size and geographical boundaries of zone), implementation, and monitoring systems, (2) the types and size of state and local fiscal incentives given to qualified EZ businesses, (3) the cost of the EZ program (e.g. state and local tax incentives, subsidies, administrative costs, additional service delivery costs), (4) the impact of EZ on business location, job creation, and private capital investments. The survey instrument contained similar questions that Lambert and Coomes (2001) used in their survey of the Louisville EZ area.

We obtained the names of the zone coordinators and participating businesses from the Alabama Department of Economic and Community Affairs (ADECA). This state agency is charged with the implementation of the enterprise zone program. The lists provided by ADECA contained the names and mailing addresses of 115 endorsed EZ businesses and 27 zone coordinators. The list of participating businesses produced by ADECA did not include a point of contact or business phone number. Therefore, we had to gather more information on each business in order to determine the appropriate individual to whom we should address the survey. In addition to obtaining the updated lists of program participants and program administrators, we visited ADECA and interviewed the state enterprise zone program coordinator. We also contacted, and were contacted by several representatives of businesses involved in the study. In order to increase a high response rate, we sent letter with the Economic Development Institute (EDI), Auburn University, letterhead with a message signed by the Director. We felt the reputation of EDI along with their ongoing relationships with state and local economic development officials would encourage businesses and particularly zone coordinators to respond to our survey.

Each business and coordinator was mailed an initial survey in May 2002. Due to an almost non-existent response rate, a second round of surveys was mailed in early July 2002. Only 65 business surveys were however mailed in the second round. It had come to our attention during the process of conducting the initial mail survey that many of the addresses provided by ADECA were inaccurate or had expired. Inaccurate and expired addresses were excluded in the second mail survey. The response rate was dismally low. We received responses from only 2 out of 115 EZ businesses (response rate—1.74%) and 1 out of 27 (3.7%) response from the zone coordinators. The two EZ businesses that returned their mail survey were Peco Foods, Inc. and Southern Engraving Inc. However, Southern Engraving aborted their attempt to complete the survey after the third question and wrote at the bottom of the first page of the survey instrument, "we don't know anything about this program." The only EZ coordinator to complete and return a survey was Billy Houston from Eufala/Barbour County.

Given the low response rate, it is not possible to draw conclusions from the survey concerning the design and the impact of the EZ program on job creation, capital investment, and business participation in the state. Accordingly, this study also utilized statistical data from ADECA's annual report, as well interviews with the state zone coordinator and several EZ businesses.

FINDINGS

This section describes the data based on ADECA's annual report on business participation, job creation, and capital investment. It then discusses findings based on the three surveys returned and interviews with the state zone coordinator and several EZ businesses. Table 2 and 3 presents cumulative data on the number of participating companies, jobs created, and capital investment in EZ counties/cities in Alabama compiled by ADECA. Table 2 reflects the data during the period of the program's start (1988) to 1995. Table 3 represents that of 1988 to 2000 for the companies that have been endorsed by local governing authorities for participation in the Alabama Enterprise Zone program. ADECA cautions readers that the agency does not guarantee the accuracy of figures reported in the data (ADECA, 2000) because the data are based on information received from participant companies and local zone coordinators. An ADECA staff member reported that he gathered annual data from local zone coordinators either through phone calls or reports sent from them to him. As such, he mentioned that great caution must be exercised concerning the reliability of the data shown here. This cautionary remark is one of the reasons we conducted the mail survey of both zone coordinators and business in order to verify the correctness of the ADECA's report. However, our effort to gain data through mail surveys failed due to a low response rate.

The information in Table 2 reveals that 17 counties/cities had actively participated in the EZ program by 1995. The number of participating EZ companies totaled 70 and their employment reached 5,111 with $757 million in investments. By 2000, as Table 3 presents, the cumulative number of participating counties/cities increased to 18 companies---a growth of only one more participating county than the previous period. The cumulative number of participating companies increased to 183, with their job creation jumping to 17,149 and private investment recording 1.6 billion dollars. This is a 161% increase in the number of participating firms, a 236 % increase in employment, and a 122 % increase in private investment from the previous period.

Table 4 presents the economic impacts of the EZ program in Alabama in two separate years, 1995 and 2000 respectively (ADECA, 1995 and 2000). ADECA's annual report is missing important data (e.g., state and local income and sales tax paid by EZ companies, estimated wages and incomes), which are crucial in assessing the impact of the EZ program. Moreover, some data are not consistent and comparable as is shown in the empty cells (N/A) in Table 4. Information in the table shows that total capital investment made by EZ companies in 1995 was $56 million and that of 2000 was $27 million. The number of jobs created amounts to 718 jobs in 1995 and 1,310 jobs in 2000. The average cost to the state per job created (taxes exempted through program/number of jobs created) decreased from $459 in 1995 to $430 in 2000. ADECA claims the program recorded $26.7 million in new income for the state after EZ exemptions in the year 2000, and thus concludes the program is cost-effective. However, the calculation of the new income by the agency is quite questionable in that the figures of estimated wages and salaries, estimated state income tax, and estimated sales tax generated, are not reported in the table. An ADECA staff member claimed he received the income figures from the Alabama Department of Revenue.

Table 2. Enterprise Zone Activity in Alabama (1988-1995)

City/County	Number of Companies	Total Jobs	Capital Investment
Birmingham	24	622	91,947,766 ($)
Montgomery	2	927	41,490,500
Butler	3	175	960,000
Clay	1	114	4,204,000
Cherokee	1	109	17,739,675
Dallas	6	275	332,262,045
Escambia	2	0	1,593,000
Etowah	6	1,367	81,537,427
Jackson	5	400	91,378,094
Mobile	1	43	2,321,000
Monroe	1	175	1,750,000
Pickens	1	83	4,500,000
Pike	7	303	43,237,704
Randolph	1	200	6,736,000
Russell	3	79	12,069,000
Sumter	5	164	22,200,000
Talladega	1	75	150,000
Totals Through 1995	70	5,111	757,076,211

Source: 1995 ADECA Annual Report

Based on Tables 3 and 4, ADECA concludes that the EZ program is effective in creating new jobs and capital investment (ADECA, 2000). The conclusion, however, is not verified by the data presented. Greater caution must be exercised in drawing conclusions. Reliable findings require that controls be applied for regional job growth and industrial concentrations in each county/city during the specific period. This procedure would determine whether the job growth is due to the EZ program or the general growth rate in the area (Dowall, 1996; Lambert and Coomes, 2001). This control can be applied by comparing the changes in economic activity between the EZ area and the non-EZ area in a county/city (shift-share analysis). In the process, descriptive statistics on employment, socioeconomic, and real estate developments to determine the extent of economic improvement in the EZ relative to other geographic areas in the local economy would be necessary (Lambert and Coomes, 2001).

Table 3. Enterprise Zone Activity in Alabama (1988-2000)

City/County	Number of Companies	Total Jobs	Capital Investment ($)
Birmingham	63	4,472	159,651,920
Montgomery	9	2,270	145,400,000
Butler	11	1,298	46,410,000
Cherokee	1	136	230,113,190
Covington	2	155	11,800,000
Dallas	27	842	352,907,000
Escambia	1	500	10,000,000
Etowah	16	2,273	160,456,589
Jackson	7	1,127	425,762,000
Lowndes	1	129	561,760
Mobile	4	1,183	21,321,000
Monroe	1	55	2,002,312
Pickens	1	75	8,241,000
Pike	8	356	52,837,704
Randolph	5	548	20028910
Russell	7	478	108,770,000
Sumter	5	164	22,200,000
Talladega	14	1,088	39,000,300
Total	183	17,149	$1,607,463,685

Source: 2000 ADECA Annual Report

In addition, a survey of employers in the EZ could be supplemented to determine whether they felt the EZ incentives were effective. Empirical studies employing shift-share analysis or multiple regressions show that the mere job growth and investment in EZ areas turn out to be negative growth once they are controlled and contrasted with the performance of non-EZ areas in the region (Dowall, 1996; Lambert and Coomes, 2001). Given the limited data available for this research in Alabama, we were unable to employ shift share analysis and multiple regressions. It is highly unlikely, however, that the EZ program resulted in significant job creation and capital investment given the poor survey response rate, poor record-keeping practices, and the use of inconsistent and questionable data in Alabama.

Table 4. Economic Impact of Alabama's Enterprise Zone in 1995 and 2000

Item	1995	2000
Total capital investment	56,496,728	27,350,000
Number of jobs created	718	1,310
Estimated wages and salaries	13,067,600	N/A
Estimated state income tax paid	457,366	N/A*
Estimated state sales tax paid	313,622	N/A*
Taxes exempted through program	329,647	563,584
Average cost to state per job created	459.12	430.22
New income for the state after EZ exemptions/benefits	N/A	26,786,416
Average hourly wage paid in zones	8.75	N/A

* ADECA admits that there is no public or local data source available to compute state and local taxes generated. Moreover, provision of this information by private source is voluntary and has not produced accurate data.

Data Source: Annual Report: Alabama Enterprise Zone Program, 1995 and 2000. Alabama Department of Economic and Community Affairs.

SURVEY RESULTS

The lone coordinator response from the Barbour County coordinator gave details about the size and geographical boundaries of that zone. The zone is located on a site known as Lakepoint Industrial Park. This industrial park is located on Highway 431 and is approximately 200 acres in size. According to the survey response, expansion opportunities do exist. The expansion would include Eufala Industrial Park and possibly some property on the north side of Eufala Industrial Park.

The implementation of the EZ program in Barbour County involves several state and local entities including City/County Chamber of Commerce, Southeast Regional Planning Commission, Alabama Development Office, and Economic Development Partnership and Utilities. The zone employs several recruitment techniques to attract businesses. Zone staff work with existing industries in expansion efforts and efforts to secure vendor leads. They also advertise through industrial recruitment program. Businesses are also referred to the zone coordinator from the state and public utilities. Qualified EZ businesses in the Barbour County zone receive several state and local incentives to locate within the zone. New industries in Industrial Park are possibly given land as well as in kind work on site preparation and the provision of utilities to the site. The local government and zone administrator also aid in coordination of all available programs such as training.

The business responses were from the Tuscaloosa and Birmingham zones. Neither had records on or knew of the types or size of incentives within their zone. Only Peco Foods Inc. gave a somewhat detailed responses to the survey. According to the responses from this business survey, the financial and technical resources supporting the EZ program can be described as fair. It seems that the lack of these resources most likely contributes to the limited understanding of the program itself by participants, as the survey response indicates. No information was provided by any of the three respondents as to the costs of the EZ program (e.g. state and local tax incentives, subsidies, administrative costs, additional service delivery costs).

The perception of EZ incentives on business location, job creation and private capital investments was mixed according to survey respondents. The Barbour County zone coordinator felt the incentives did effect business location and expansion in his area. However the response from Peco Foods indicated the incentives played a minimal role in their initial decision to locate (1987) but such factors would play a larger role now. Peco Foods also indicated they had made 5 million dollars in capital investment since 1987 but did not indicate whether zone incentives had an influence on those investments. They indicated that their firm had plans for expansion and that the EZ incentives would be a factor in that expansion.

DISCUSSION

Although the annual EZ reports prepared by ADECA conclude that the Alabama EZ program as a whole is a cost efficient tool for promoting economic development of distressed areas in the state (ADECA, 2000), the survey results and interviews with zone coordinators and businesses indicate there is high probability that any growth within the enterprise zone is not directly attributable to EZ program. Major problems associated with the zone design, implementation, monitoring, and evaluation are examined in this section. Survey results and interviews show that the major problems that have hampered the effective use (operation) of the EZ program in the state are: poor record keeping practices, a lack of communication, a lack of professionalism, and poor resources (staff, financial and technical).

First, poor record keeping practices on the part of state agencies, local zone coordinators, and participating businesses were prevalent, which hampered our effort to explore and evaluate the effectiveness of EZ programs in Alabama. Good record keeping practices are a prerequisite for an ongoing as well as a comprehensive evaluation of any economic development program. Sound data are required to determine the efficiency and effectiveness of such programs. Although the survey responses were extremely limited in both the number and quality of information, the three responses secured as well as interviews with state officials and phone interviews with non-responding businesses show that record keeping on the part of all involved with the program is lacking. A common response when asked for specific numbers associated with program incentives was "no records". The business survey response cited that there was no requirement by law to submit annual reports to the zone coordinator. The lone responding zone coordinator also indicated that he did not submit an annual report to ADECA as the enacting legislation requires (Code of Alabama. 41-23-23). If in fact earlier growth in zones was stimulated by the program, the poor record-keeping practices by the parties involved make it impossible to establish relationships.

Second, a lack of communication between participating businesses and zone coordinators was clearly revealed in the study. The lone business survey response indicated that there had been no communication between the coordinator and the business. However, the Barbour County zone administrator mentioned that he communicated "as often as possible" with participating businesses. Unfortunately, this can not be proven since there was no business response from the county. It should be noted that in order for the program to be smooth and effective, ongoing communication among parties involved in the program is a must.

Third, a lack of professionalism among state and local coordinators seems to be a big hurdle for the effective and efficient operation of the program. Specifically, the lack of professionalism was clearly evident on the part of individuals working within ADECA and the Alabama Department of Revenue. We made numerous calls, as well as sent emails, to contact the state EZ coordinator. However, the state coordinator made little or no effort in returning our calls. Visiting with ADECA staff were not productive. ADECA staff was not able to provide the information we needed. After several attempts, the state coordinator finally provided a list of businesses, but did so with the disclaimer that not all of the businesses on the list were participants. The list contained all the businesses that were approved for participation, but did not distinguish actual participants from those that were merely endorsed. He left the matter of making those determinations to us. It is essential to maintain an accurate and up to date list of participating EZ businesses and local zone coordinators to successfully administer the program. Unfortunately, state officials charged with this responsibility have failed to maintain appropriate records.

Fourth, inadequate staffing and poor resources (financial and technical) also played a role in the ineffective operation of the zone program. During the course of the evaluation we had several opportunities to speak with the state program coordinator as well as approximately 8 participating businesses. The state program coordinator informed us that he only devoted about 5% of his time to the program and that participants (businesses) had lost interest in the program over the last 3 to 5 years. The loss of interest by program participants was also reflected in several telephone conversations between us and businesses. Several of the businesses listed as participants contacted us to inform that they had not participated in the program for some time and did not have any information to provide with regard to the period in which they were active participants. We also had phone conversations and email correspondence with representatives of several businesses listed by ADECA who did not know what the Enterprise Zone program was and claimed to have never participated in the program. One business respondent claimed that one of the reasons businesses lost interest in the zone program was insufficient financial and technical assistance given to businesses by state and local agencies.

Having identified several deficiencies associated with the Alabama Enterprise Zone program, an examination of cases in other successful states will provide additional insights to the state of Alabama. The case of the California Enterprise Zone program is an example of such a program. Although the population size and the economic situation of California and Alabama are quite different, a comparison of the fundamental aspects of zone design, implementation, and evaluation processes can be beneficial to the state of Alabama.

The Enterprise Zone program in California began two years earlier (1985) than Alabama. Since then, 39 EZs have been established in the state. These EZs provide similar tax and non-tax breaks to those found in Alabama to businesses that locate in the zone (Dowall, 1996). The California's enterprise zone program is overseen by the Technology, Trade and

Commerce Agency (TTCA). Unlike Alabama, however, California formed a professional association (California Association of Enterprise Zones--CAEZ) in which state and participating businesses cooperate to find ways to enhance the overall effectiveness of the zone program. Since 1990, the TTCA has implemented EZ programs in coordination with CAEZ. The CAEZ is a non-profit organization served by an eleven-member board of directors who have vast experience in the EZ programs. The mission of the CAEZ is to: 1) advance the art and science of implementing economic development in EZs, 2) initiate and prepare legislative action designed to enhance or preserve EZs, 3) develop specific written positions on state and federal legislation and administrative policy affecting economic development in EZs, 4) foster the interchange of ideas and educational experiences in the field of EZs, and 5) enhance the career growth of professionals employed in the field of economic development in EZs (California Association of Enterprise Zones, 2003).

To achieve the above goals, the CAEZ has implemented the Zone Manager Certification Program in coordination with TTCA in order to enhance the competency and professionalism of zone coordinators (California Technology, Trade and Commerce Agency, 2002). There are nine classes in this certification program, including five core and four electives. Some of the areas covered in these classes include; vouchering, annual report performance measure, audit preparation and tax credits. The program ensures that the individual charged with coordinating zone activities is fully capable of handling the needs of participating businesses. In addition, in order to maintain smooth and effective communication between businesses and the zone coordinators, workshops are utilized through which businesses are provided with technical assistance and other necessary information.

Another technique used by California is to formally survey customers and constituents. This survey allows program administrators to make determinations about their satisfaction with current assistance, as well as to identify any unmet needs. A zone manager certification program, workshops, professional associations, and customer survey similar to California would aid in providing assistance to Alabama's zone program participants. Such efforts would increase program knowledge as well as information regarding the technicalities related to the successful implementation of the program. The close coordination between TTCA and CAEZ in California has facilitated the effectiveness of California EZ program in creating jobs and investments (O'Keefe, 2003; Bradshaw, 2003).

As indicated, Alabama Enterprise Zone program appears to have minimal communication with other state agencies, local zone managers and participating businesses. Nor does there appear to be any use of customer satisfaction surveys. This being the case, the program has little chance of being run efficiently or effectively.

CONCLUSION

This study has attempted to assess the effectiveness of the zone design, implementation, and economic impacts of the enterprise zone program in Alabama by using data from mail surveys as well as annual reports published by ADECA. Poor survey response rates both by EZ coordinators and businesses, poor record keeping practices by state and local EZ coordinators, a lack of professionalism by state and local coordinators, as well as difficulties in accessing government data made it very difficult to produce any meaningful findings. Although ADECA's annual report claims an increase in employment and capital investment due to the EZ

program in the state, such claims seem to be undocumented and unfounded at best. There is a lack of supporting records and inconsistent data. The study also shows that further avenues for future research on the EZ program in Alabama are limited as long as the state and local officials and participating businesses continue to maintain poor record keeping practices and do not respond to surveys and requests for information.

Several suggestions can be made to improve the response rate of survey instruments related to this and similar studies in the future. First, the number and proportion of open-ended questions may be minimized while that of multiple-choice questions could be increased. It is probable that the businesses which received the EZ survey were reluctant to complete the survey due to the large amount of information requested regarding zone design, implementation, financial incentives, personnel, and performances. Although questions regarding the amount of incentives, capital investment, and the number of employees were intended to assess the numbers presented by ADECA's annual reports, these questions could have created an inclination not to respond to the survey. It should be noted, however, that it is crucial to get figures concerning financial and employment data in order to reach meaningful findings. A heavy reliance on multiple-choice questions regarding these figures would limit the scope and magnitude of such research and would be superficial at best. It should also be mentioned that EZ survey response rates in other states such as New Jersey, California, and Kentucky ranged from 20 to 60% and that researchers used survey instruments similar in format as used this study (Lambert and Coomes, 2001). In these states, researchers found that government agencies charged with EZ projects and businesses cooperated when asked for data. Although the low response on the part of the Alabama businesses can possibly be attributed to the design of the survey instrument, the low response from Alabama zone coordinators is more problematic. It seems that the traditional political culture within the state (Thomas and Stewart, 1988) and the lack of professionalism among state and local officials are likely the reasons for the disregard of our survey by state and local zone coordinators. It should be noted that citizens' distrust against politicians, public officials, and anti-tax sentiment has been demonstrated recently by the two-to-one rejection of Governor Riley's tax increase and accountability proposal. In addition, a recent study shows that many state employees lack the necessary professional training and background to develop the state to its maximum potential (Barret et al. 2003).

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AN INNOVATIVE METHOD TO FUND NEW CHEMICAL INSTRUMENTATION AND INCREASE LABORATORY SAFETY WITH APPLICATIONS FOR STATE TWO AND FOUR YEAR COLLEGES AND UNIVERSITIES IN ALABAMA

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ABSTRACT

An innovative method of obtaining funding for new chemical instrumentation is described which has been successfully implemented by the Department of Physical Sciences at Auburn University at Montgomery. The same method might well be implemented by other Chemistry or Physical Science departments in Alabama to their advantage.

INTRODUCTION

Two issues that greatly concern the majority of physical science and chemistry departments are the availability of modern (especially computer controlled) instrumentation and chemical safety, particularly for students, in the chemistry laboratories. We believe that, at Auburn University at Montgomery(AUM), we have found several innovative methods over the past several years to alleviate these two concerns. It is the purpose of this paper to describe these methods which might be emulated to their advantage by other two and four year colleges and universities in Alabama that teach chemistry . The methodology described here has applications in physics, physical science and biology laboratories as well.

HISTORICAL PERSPECTIVE AND PROJECT L.A.B.S.S.

Some years ago this Department received U.S. Department of Education funding for Project L.A.B.S.S. [Laboratory Adaptations for the Betterment of Special Students](McDaniel et al., 1994) which sought to increase the number of students with physical disabilities (particularly those who used wheelchairs) who took General Chemistry classes, which are generally a requirement for entry into Professional Schools, such as Pharmacy or Medical schools. One of the

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main objectives of the Project was to produce a laboratory manual for use in the General Chemistry sequence at AUM where the experiments described therein were suitable for a person who was in a seated position at a table.

This person might or might not have been using a wheelchair in the laboratory. The experiments therefore had to have a very high degree of chemical safety. The usual operating paradigm in Project L.A.B.S.S. was that the entire experiment could be spilled on to the student's lower body without any *chemical* consequences, such as burning from hot chemical or corrosive materials, even though the person might become wet or cold or possibly a little humiliated.

Project L.A.B.S.S. did indeed develop a laboratory manual (McDaniel et al., 1996) containing twenty-four experiments where no chemical principle normally taught in General Chemistry laboratories was compromised, but also used the following general safety criteria:

1. All chemicals had to be available from supermarkets or drugstores on the open market, not from chemical or scientific supply houses, which meant that the experiments were highly cost effective and could be performed even outside conventional chemistry laboratory settings. (Teggins et al., 1998; Mahaffy et al., 1999).

2. No temperatures above 50 degrees Celsius were permitted.

3. Only very weak acids or bases, of the order of 0.001 molar were permitted. For example, muriatic acid is available for use in swimming pools in retail stores, but was not used in Project L.A.B.S.S. without considerable dilution.

4. No open flames were permitted.

5. The developed procedures should generate as little waste as possible, which would help to lower the disposal costs and lessen the environmental impact associated with performing the experiments.

This manual was provided free of charge to ALL students in the General Chemistry sequence at AUM for a period of five years after the termination of the Department of Education grant, which was part of the agreement between AUM and the U.S. Department of Education at the inception of the grant. A few of the more novel experiments were described in the chemical education literature.(Mahaffy et al., 1997; Teggins et al., 1998)

Although Project L.A.B.S.S. was a success (McDaniel et al., 1994; Rawls, 1997) in that it accomplished its dual goals of producing the laboratory manual using the criteria mentioned above, and also increasing awareness of students with disabilities pursuing courses, majors, and professional careers involving chemistry both from the student and faculty perspectives, (Mahaffy et al., 1995) a few drawbacks of the Project L.A.B.S.S. philosophical approach to teaching General Chemistry emerged during the five year post-grant laboratory trial period. The first was that we did not have a single application at AUM from a student with a physical disability who wished to pursue a career in a professional field that involved a required General Chemistry course,

although we did have a large number of inquiries for assistance from other institutions who were assisting students with disabilities in their programs, both from within the U.S. and from abroad, particularly from Canada. Because of this perceived or otherwise paucity of enthusiasm, the faculty teaching General Chemistry at Auburn University at Montgomery began to (correctly) question why ALL of our students had to take the Project L.A.B.S.S. version of the General Chemistry laboratory, which some regarded as being somewhat artificial, where other General Chemistry students in other colleges and universities who were competing for a limited number of places in professional school did not have these limitations placed upon their general chemistry laboratory experiences. It would have been quite feasible, in their view, to allow any student with a physical disability to take the Project L.A.B.S.S. version of the General Chemistry laboratory, while non-disabled students had a somewhat different laboratory experience. Indeed, it so happens that the Laboratory Coordinator of the chemistry laboratories at AUM has a physical disability and he and the whole Department would have been very aware of the obstacles a student with a physical disability would face, and we would all have been very empathetic to any such student.

This is, of course, a major philosophical deviation from the original Project L.A.B.S.S. criteria, but given the lack numbers of students with disabilities registering for our General Chemistry laboratories, it became difficult to continue to disagree with the views of the faculty members mentioned above.

INNOVATIVE FEATURES OF THE NEW GENERAL CHEMISTRY MANUAL DERIVED FROM THE PROJECT L.A.B.S.S. MANUAL

The chemists in the Department of Physical Sciences at AUM then agreed to write a new laboratory manual, now in its third edition (Arnold et al., 2002) which would include many of the best features and experiments of the old Project L.A.B.S.S. manual, but would address the concerns of the faculty concerning the 'reality' aspects of some of the old experiments and which also had some innovative new features. Tables 1 and 2 give the list of experiments of the new manual which readers will note covers all the normally accepted topics covered in a freshman General Chemistry laboratory manual.

The original Project L.A.B.S.S. manual had been commercially duplicated using funds from the grant, but we decided in the case of the new manual that we would duplicate the manual using the services of the print shop operated by the Alabama Correctional Industries as they offer printing services to State Institutions at a considerably lower cost than do commercial printers. We then sell the manual through the AUM Bookstore, putting the funds generated after paying for the printing services into an account to be used for the acquirement of either additional safety equipment for the chemistry laboratories, or for new or additional computer controlled chemical instrumentation. Apart from the obvious safety and fiscal advantages to the Department, there are other advantages as well. The experiments have been specifically written for the equipment that is available at AUM, check in and safety sheets that each student must sign before beginning the laboratory can be included in the manual, and we also used 'tear out' pages which the students use to record their original data, which must be handed in together with the rest of their written laboratory reports. These sheets help to alleviate the post-laboratory 'massaging' of their data. However, after these 'tear out' sheets have been used, the laboratory manual obviously cannot be

reused by another student. We reprint these manuals every year which also allows us an opportunity to modify experimental procedures or add new experiments. The pages are not numbered sequentially, pagination being in the form 'Experiment 1-1 to 1-5' for a five page experimental procedure which greatly facilitates the removal or addition of new or modified experiments.

There are also considerable advantages to the student. First, the manuals can be sold to the student at a price considerably less than the equivalent commercially available manual for general chemistry, and our manual includes check-in sheets and safety information specific to the laboratories at AUM. Second, instructions for the operation of specific pieces of software or spectrometers can be included for the exact make and model of equipment which the student will actually use in the laboratory rather than generic instructions which are usually found in commercially available manuals. The writers of these manuals and the Department of Physical Sciences at AUM obviously hold the copyright on this publication, and on all the other manuals mentioned below. They may not be duplicated by any other person or organization without the expressed written permission of this Department. Tables 1 and 2 give the titles of the experiments used in the manual:

Table 1. List of Experiments for the First Semester of General Chemistry

1. Chemical Laboratory Safety
2. Units and Measurements
3. Density of a Solid: Graphical Method
4. Paper Chromatography
5. Stoichiometry: Determination of a Chemical Formula
6. Preparation of a Copper Compound
7. Analysis of Unknown Acids by Acid/Base Titration
8. Determining the Ideal Gas Constant
9. Specific Heat of Antifreeze
10. Enthalpy (Heat) of Solution (NH_4NO_3)
11. Flame Tests

Table 2. List of Experiments for the Second Semester of General Chemistry

1. Analysis of acetic acid in vinegar
2. Chemical kinetics
3. Colorimetry: determination of copper ion in solution
4. Determination of an equilibrium constant
5. Determination of k_a and pk_a of weak acids
6. Electrochemistry : galvanic and electrolytic cells
7. Introduction to the spectrophotometer
8. Kinetic order of a reaction
9. Observations of reactions
10. pH of acid, base, and salt solutions
11. Preparation and analysis of alum
12. Preparation of and analysis of a coordination compound : potassium trisoxalatoferrate (III)
13. Reactions of ionic copper

OTHER LABORATORY MANUALS

The Department has also just published the third edition of a simplified version of the manual used in the General Chemistry sequence for use in our non-major Introduction to Chemistry course, (Arnold et al., 2002) which uses the same framework as the General Chemistry manual described above. Table 3 gives the list of experiments:

Table 3. List of Experiments for the Non-Major One Semester Introduction to Chemistry

1. Chemistry Laboratory Safety
2. Measurement
3. Density of a Solid
4. Heat of Reaction (ΔH)
5. Stoichiometry
6. Reaction Observations
7. Paper Chromatography of Ink
8. Flame Tests
9. Molecular Models
10. Hydrolysis Constant For The Carbonate Ion
11. Acid/Base Titration
12. Chemical Kinetics
13. Specific Heat of Antifreeze
14. Boyle's Law
15. Colorimetry: Determination of Copper Ions in Solution
16. pH of Acid, Base, and Salt Solutions
17. Analysis of Acetic Acid in Vinegar

A third manual for use in our non-major Introduction to Physical Sciences course is also in its third edition (Arnold et al., 2002) again using the same tenets as the other two. The topics covered are in this manual are given in Table 4:

Table 4. List of Experiments for the Non-Major One Semester Introduction to Physical Science course

1. Measurement
2. Linear Motion
3. Projectile Motion
4. Mass, Weight and Gravitation
5. Simple Machines
6. Calorimetry
7. Reflection and Refraction
8. Ohm's Law and Series and Parallel Circuits
9. Magnetism
10. Spectroscopy
11. Dimensions of a Molecule
12. Lenses and the Lens Equation
13. Radioactivity

A new laboratory manual which covers both semesters of General Physics has more recently been prepared (Arnold, 2001) the contents for which are given in Tables 5 and 6.

Table 5. List of Experiments for the First Semester of General Physics

1. Vectors and Forces
2. Equilibrium
3. Linear Motion, Velocity and Acceleration
4. Work, Simple Machines and Friction
5. Uniform Circular Motion
6. Hooke's Law and Simple Harmonic Motion
7. The Ideal Gas Law and Boyle's Law
8. Specific Heat Capacity

Table 6. List of Experiments for the Second Semester of General Physics

1. Resonance and the Speed of Sound in Air
2. Reflection and Refraction
3. Optics and Lenses
4. Interference and Diffraction
5. Electrical Field Mapping
6. Ohm's Law and Series and Parallel Circuits
7. Capacitance and the RC Time Constant
8. Magnetism
9. Induction

Individual faculty members in the Department of Physical Sciences are currently working on further manuals which will cover the Analytical Chemistry (Thomas, 2004), Biochemistry (Rawlings, 2003), and Introduction to Astronomy (Russell, 2004) courses at AUM.

If one analyzes the processes outlined above, the faculty in the Department donated their time and expertise to the development of these manuals, but instead of obtaining royalties from their publication, they donated their services to the Department to the betterment of all those in the Department whether they be students or faculty. One might argue that these types of measures should not be necessary in a properly funded educational situation, but in the particular case of Alabama, the reduced revenues from State Sales Taxes have effectively decreased Operational and Maintenance budgets a great deal in constant dollar terms over the last decade, as is also seen in the percentage of the total amount of fees the student has to bear today as compared to a decade ago. The funding of state of the art scientific equipment has never been a priority when an older model which ostensibly 'does the same thing' is currently available, not withstanding that if the student eventually obtains a position in a modern chemical industry, they will be required to exhibit competence on state of the art instrumentation.

There is no reason whatever why our methods outlined above for obtaining additional funding for sciences departments in the State of Alabama or any other state may not be emulated by other schools or universities. It is true that there was a considerable expenditure of effort on the part of all members of the Department to prepare the original copies of the various manuals, but once this has been achieved, the continued updating and annual printing does not require a large amount of time. Without these projects having been completed in the last few years, our students

would not have the access to state of the art equipment and our laboratories would not contain the latest safety equipment. It would seem to the writers that this type of project might be worthy of consideration by many other schools that found themselves in the predicament that we found ourselves in before the inception of the processes described above.

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RELATIONSHIP VIOLENCE AMONG AFRICAN-AMERICAN COLLEGE STUDENTS

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ABSTRACT

It has become increasingly evident that violence in intimate relationships is not limited to married couples but is also experienced during the dating and courtship phase among college students. Despite the increased focus on intimate relationship violence, only a few studies explore the negative consequences of dating violence. This study explores the prevalence and consequences of dating and courtship violence among 215 African-American college students. This study examines the range of interpersonal problems with work/school and self and their relationship to dating and courtship physical and psychological abuse. The results show that African-American students are more likely to experience psychological abuse than physical abuse and the negative impact from such abuse is consistent with that previous research findings; experiencing depression, stress and anxiety and long and severe headaches.

REVIEW OF LITERATURE

More than two decades of research show intimate relationship violence as a major social problem for women of all cultures, races and income levels. It has become increasingly evident that violence in intimate relationships is not limited to married couples, but is also experienced during the dating and courtship phase among high school and college students. A review of available statistics illustrates the magnitude of the problem. One-third of all female homicide victims are killed by their husbands, ex-husbands or boyfriends as are murdered by strangers. In a study of females killed by intimate partners between 1980-1982, Stout (1991) found that the majority of women killed were married (57.7%, n=2,415); girlfriends were the next highest percent (24.5%, n=1,041) followed by common-law wives (8%, n=332), ex-wives, (4.9%, n=205 and friends (4.6%, n=196). The number one killer of African-American females, ages 15 to 34, was homicide at the hands of an intimate partner or ex-partner. According to FBI statistics, 30% of female murder victims are killed by their husbands or boyfriends (Sullivan and Rumptz, 1994). Estimates of dating and courtship violence among

high school students range from 12% to 48%. In a survey of students (N=644) in five Midwestern high schools, 12.1% (N=78) of the high school students reported involvement in a violent dating relationship (Henton, et al., 1999). In Johnson-Reid and Bivens'(1999) needs assessment survey of dating violence among youth in foster or group care, 48% of these youth reported involvement in some dating violence. Studies of courtship violence among college students show rates of students involved in violent relationships ranging from 16.7% to 78% (Makepeace, 1999; Harned, 2001). Makepeace's (1999) study of 2,338 students attending seven Midwestern colleges found 16.7% of the students reported violence in the courtship experience. A study of 874 college students engaged in the dating experience found students reporting both psychological (82% of women and 87% of men) and physical abuse (29% of women and 21% of men) from dating partners (Harned, 2001).

The literature that addresses the negative consequences of youth dating is limited, however, youth dating violence is found to mirror that of adult domestic violence (Sousa, 1999). Studies that explore the physical consequences of adult battered women show loss of work hours and medical cost as the most obvious and visible consequences of abuse by an intimate partner. Shepard and Pence (1988) found a high rate of absenteeism among abused women. An analysis of data from the National Violence Against Women Survey (Tjaden and Thoennes, 1998) revealed that in one year hospital emergency facilities treated 546,900 female physical assault victims. Medical costs from domestic violence are estimated to total from three to five billion dollars a year (Miller, et al., 1996).

Battered women are also found reporting a wide range of psychological problems. Major depression, alcohol and other drug abuse, anxiety, anger, shame, lowered self-esteem, and impairment in other areas of functioning are some of the psychological consequences reported by victims in abuse relationships (Arias and Pape, 2001; Dutton, Goodman and Bennet, 2001). For example, when Follingstad and others (1991) used a checklist of 12 physical and psychological symptoms to identify the range of problems among 234 physically abused women, a majority (65%) of the sample reported three to five of the symptoms. Depression was the most frequently cited symptom (77%) followed by anxiety (75%) and persistent headaches (56%).

Research that focuses on outcomes of psychological and sexual, in addition to physical abuse in intimate partnerships indicate that psychological abuse within the context of intimate relationship may be as damaging if not more damaging than physical abuse. Shepard (1991) believes that emotional abuse attacks the partner's self-worth and makes the victim feel as if the abuse is deserved. A majority of the physically abused women in Follingstad survey (1990) stated that the psychological abuse of their partners impacted them more negatively than the physical abuse. The findings of Harned's (2001) survey of undergraduate and graduate students indicated that psychological abuse can have negative psychological and school-related consequences. The psychological and sexual abuse from the intimate partners of these subjects were significantly related to negative psychological outcomes (depression, anxiety, posttraumatic stress, and body shape concern). Arias and Pape's research (2001) found psychological abuse to be a strong and significant predictor of posttraumatic stress disorders symptoms among 68 women in a battered women's shelter in Georgia. Subtle psychological abuse predicted more detrimental effects on the 834 women in Marshall's (2001) student than did overt psychological abuse, violence and sexual aggression.

THE RESEARCH PROBLEM

Despite the increased focus on intimate relationship violence among youth, only a few studies explore the negative consequences of dating violence. Even fewer studies have examined the complexities of dating and courtship violence among African American youth. This study explored the prevalence and consequences of dating and courtship violence among African American students at a predominantly African-American university in the south. Dating and courtship violence is broadly conceptualized to include incidences of psychological (nonphysical violence), sexual and physical acts of aggression. The study examines the range of physical, interpersonal, psychological and work/school related problems that are found associated with dating and courtship violence.

THE SAMPLE

The sample consists of 215 students attending a predominately African-American University in the southern region of the U.S.; the sample is 66% female. The university has an enrollment of approximately 6,000 students. The majority of the sample (71%) is single and 22% are in long-term relationships with a male or female. Ages range from 18-43, with an average of 23.6. Three-fourths of the sample is from within the state, with 11% from the southeast and southwest, 8% from the northeast and less than one percent from the northwest. In response to the size of residential area: 22 percent are urban; 41 percent, rural; and 30 percent, from a small town. Most of the students (78%) live on campus.

METHODS

A questionnaire exploring personal experience with physical and emotional abuse, feelings about self as a person and outcomes of violent episodes was administered to college students. In addition to descriptive variables such as age, gender, income, education, marital status, number of years with spouse/partner, racial and ethnic background and place of residence. Three scales were administered: the Global Screen Inventory (GSI), the Partner Nonphysical Abuse Scale and the Partner Physical Abuse Scale. Students were asked how they responded to situations of physical or non-physical abuse.

The **Global Screening Inventory (GSI)** may be described as a multidimensional assessment scale. The GSI is multidimensional at the item level, which means that each item is its own subscale. The GSI is designed to obtain a very rapid evaluation of the individual's problems across 18 different areas of functioning. The Global Screening Inventory scale is a brief index of the extent to which someone may be having problems in one or more personal or social areas. The GSI is a problem checklist in which each of the items has been scaled according to a relative frequency continuum. Thus, although the total score obtained from the GSI will be of some use, its principal application is to examine the responses to each of the individual items.

Each item on the GSI is scored using a scale from 1-7; with 1=none of the time and 7=all of the time. Any item score of 3 or less indicates there is probably no clinically significant problem in the area, while scores of 4 or greater are generally indicative of problems with which the client could likely benefit from professional help.

The principal weakness of the GSI arises from the fact that a person can easily obtain

a very low score like having serious problems in a single area of personal or social functioning. Because of this feature, it is doubtful that the GSI will ever have a meaningful clinical cutting score. Suppose, for example, that an individual has no difficulties in any of the areas covered by the GSI except for problems with co-workers on the job. That problem could be so severe that the individual is on the verge of being fired from his/her job. Yet the score on the GSI scale could be very low. On the other hand, if the respondent does have a very large total score on the GSI, that nearly always indicates a multi problem situation. Larger scores do suggest a more severe and more numerous set of respondent problems, yet because single problems can get lost in the total score, it is important to pay close attention to item responses (Nurius and Hudson, 1993).

Partner Abuse Measures

Two measures are designed to assess the degree of physical and nonphysical abuse that is exchanged between partners. The concept of abuse takes many forms, and for our purposes it is conceived in the broadest terms as various forms of misuse or ill-treatment. The following scales distinguish between two kinds of abuse: physical and nonphysical. Physical abuse consists of those behaviors in which one partner strikes the other, sets about to inflict bodily harm, or sets forth an extreme threat of such harm. By comparison, nonphysical abuse consists of behaviors that assault the individual's integrity or persona, are designed to limit freedoms, or cause unreasonable deprivation. In a sense, nonphysical abuse consists of ill-treatment that do not fall within the definition of physical abuse (Nurius and Hudson, 1993).

The **Partner abuse Scale-Nonphysical** measures the degree of perceived nonphysical abuse that an individual receives from a spouse or partner. The nonphysical scale has been partially validated, it has reliability of .90 or greater, good content, factorial, and construct validity (Nurius and Hudson, 1993). At present a known clinical cutting score is not available, but research is being conducted in an effort to obtain that information.

Subjects were asked to respond to the following 18 items about the extent of non-physical abuse-- My current or past partner:

1. Has belittle me
2. Has demanded obedience to his or her whims
3. Has become angry if I say he or she is drinking too much
4. Has become very upset when the house was not clean as he or she thought it should be
5. Did not want me to have any male/female friends
6. Has called me ugly and unattractive
7. Acted as if I was his or her personal servant
8. Has insulted or shamed me in front of others
9. Became angry if I disagreed with his or her point of view
10. Belittled me intellectually
11. Demanded that I stay home
12. Felt that I should not work or go to school
13. Screamed and yelled at me
14. Shouted and screamed at me when he/she was drinking
15. Ordered me around
16. Has no respect for my feelings

17. Said that I really couldn't manage or take care of myself without him or her
18. Become angry if I disagreed with his or her point of view

The **Partner Abuse Scale-Physical** measures the degree of perceived physical abuse that a client receives from a spouse or partner. This instrument has been partially validated, it has a reliability of .90 or greater, and it has good content, factorial, and construct validity. At present a known clinical cutting score is not available, but research is being conducted in an effort to obtain that information (Nurius and Hudson, 1993).

The 12-Item Physical Abuse Scale asked the following questions of a current or past partner:

1. Has physically forced me to have sex
2. Has pushed and shoved me around violently
3. Has hit and punched my arms and body
4. Has threatened me with a weapon
5. Has beat me so hard that I sought medical help
6. Has slapped me around my face and head
7. Has beat me when he or she was drinking
8. Has threatened to cut or stab me with a knife or other sharp object
9. Has tried to choke or strangle me
10. Has bitten or scratched me so badly that I bled or bruised
11. Has violently pinched or twisted my skin
12. Has injured by breasts or genitals

RESULTS

Global Screening Inventory

The Global Screening Inventory (GSI) consists of 18 items assessing interpersonal problems, stress and anxiety, thoughts of harm to self or others, drug use, feelings of guilt and the prevalence of severe headaches. Respondents who indicate they experience the behavior "some of the time," "a good part of the time," "most of the time" or "all of the time," are included in the table. Responses of "a little," "very rarely" or "none at all" are excluded from these results. The 18-items scale is categorized as the following: **Interpersonal Problems .** For this sample, 22.3 percent have problems with family members, 20.9 percent have problems with a partner; 15.8 percent have problems with the job or schoolwork and 10.2 percent have problems with co-workers. Only 5.2 percent have problems with friends. **Stress and Anxiety.** The results show that 33.1 percent feel intense stress, anxiety or nervousness; 20.3 percent feel that they are not as good as other people; 26.0 percent feel depressed blue or despondent and 10.2 percent feel intense fear or terror. **Thoughts of Harm.** Less than 2 percent thought about taking their own life or about hurting someone in the family; however, 7.4 percent think about hurting someone outside the family. Approximately 10 percent feel others are trying to embarrass or harm them (9.2 percent). A similar percentage (8.8) feel intense shame or guilt. **Drug Use.** About 13 percent drink to intoxication and 3 percent use other drugs to get "high." **Physical Problems.** Respondents were asked how often they experience severe and prolonged headaches: 14.9 percent experience headaches "a great deal

of the time."

For the Global Screening Inventory Scale response scores range from "none of the time" =1 to "all of the time" =7. The 18 item scale scores range from 18 to 126 points. While 46.6 percent of the sample scored low, indicating a response of never, very rarely or a little of the time, 53.3 percent indicate a response of some of the time or a good deal, most of the time or all of the time. Each item however must be evaluated based on its own merit. For example, perhaps an individual will not experience a problem with interpersonal interactions, yet thinks about hurting himself or herself a great deal of the time. Table 1 shows the results of the GSI, non-physical abuse and physical abuse.

Non-Physical Abuse Scale

Respondents were asked to respond to 18 items about the extent of non-physical abuse. Response categories ranges from "never" (1) to "all of the" (7); scale score ranges from 18-126. Over half of the sample (54.9 percent) fell in the "medium" range, indicating a response of very rarely, a little or some of the time; 36.2 percent scored "low" indicating the problem does not exist and 8.8 percent scored "high" indicating the problem exist a good part of the time, very frequently or all of the time.

Physical Abuse Scale

The Physical Abuse Scale consists of twelve items. The scale was scored the same as the non-physical abuse scale; scale scores range from a low of 12, to a high of 84. The majority of the sample (70.0 percent) did not experience problems with physical abuse; however 22.4 percent experienced "medium" physical abuse and 6.6 percent experienced "high" physical abuse.

Global Screening Inventory, Non-Physical and Physical Abuse

A Chi-Square test was used as a benchmark to examine the relationship between the GSI, non-physical abuse and physical abuse (Table 1). A statistically significant relationship exists between GSI and non-physical abuse. Among students scoring" low" on the GSI, 63.0 percent have experienced no or little non-physical abuse, 36.9 percent experience "some" and 37.5 percent a "high" degree of non-physical abuse. Among student scoring "medium" on the GSI, 36.9 percent have experienced "no or little" non-physical abuse, 63.0 percent experience "some and 62.5 percent have experienced a "high" degree of non-physical abuse. There is a tendency for those scoring high on the GSI to experience a higher incidence of non-physical abuse than those who score low. When examining GSI and physical abuse, chi-square was not significant; however, there is a tendency toward significance (p. <10), indicating that a higher GSI score is associated with a high incidence of physical abuse.

Table 1. Global Screening Inventory, Non-Physical Abuse Scale and Physical Abuse Scale

GSI	Non-Physical Abuse			Physical Abuse		
	Low	Medium	High	Low	Medium	High
	N %	N %	N %	N %	N %	N %
Low (18-39)	46 (63.0)	41 (36.9)	6 (37.5)	68 (50.0)	15 (34.8)	3 (27.3)
Medium (40-49)	27 (36.9)	70 (63.0)	10 (62.5)	10 (62.5)	28 (65.1)	8 (72.7)
Total	73	111	16	136	43	11
$X2 = 12.60$ $P < .001$				$X^2 = 4.56$ $P < .10$		

Response to Non-Physical and Physical Abuse

The responses to physical violence when the partner said that he was sorry were: (1) to break off the relationship (9.8 percent); (2) to continue the relationship (7.0 percent) or (3) to be have as though it never have happened (70.0 percent); see Table 2. Respondents were more likely to continue the relationship (14.9 percent) than to break-off the relationship (7.0 percent) when the partner insulted, shamed or belittle them than if the partner used physical force. Among those experiencing physical abuse, 3.7 percent believed it was their fault (Table 3). When experiencing non-physical or physical abuse, students are more likely to confide in a friend (31.2 percent), a close relative (18.6 percent) and less to an agent of law enforcement (14.9 percent), a minister (13.0 percent) and even less to a counselor (3.3 percent).

DISCUSSION AND CONCLUSION

It has become increasingly evident that violence in intimate relationships is not limited to married couples but is also experienced during the dating and courtship periods among high school and college students. FBI statistics show that women tend to be murdered by intimate others, and girlfriends ranked second in this area, with married women ranking first (Stout, 1991); numbers change with African-American females. The number one killer of young African-American females is an intimate partner or ex-partner (Sullivan and Rumptz, 1994). Studies of partner violence among college students show the incidence of violence ranged from approximately twenty to eighty percent (Makepeace, 1999; Harned, 2001).

Research findings show that both physical and psychological battering are harmful to the victims (Harned, 2001); the results of this survey tends to support those findings. In reports of interpersonal and social problems, students report experiencing (1) stress, anxiety, nervousness and depression; (2) problems with family members, (3) problems with partner; (4) job or school work; and (5) severe and prolonged headaches. A smaller percentage of students experience problems with friends, had thoughts of suicide, hurting someone in or outside the family, had problems with use of drugs other than alcohol, which tends to be the drug of choice when confronted with problems. However, even if one person experiences any of the above problems it is nonetheless important for that individual even though a significant percentage of the population is not experiencing it. The results of the survey tend to support those of previous research on the relationship between non-physical or psychological abuse and personal and social problems (Arias and Pape, 2001; Dutton, et al, 2001; Follingstad, et al, 1991). Students report experiencing minor to moderate non-physical or psychological abuse from a former or current dating partner to a greater extent than physical abuse. There is a strong tendency for the findings of this research to support that of previous studies on the relationship between physical abuse and personal and social problems.

Women often experience their greatest risk of violence not from acquaintances and strangers but from their intimate male partners. The image of a woman being abused by those who claim to love and honor her is horrifying. However, such abuse is one that many of us have heard of or, perhaps, experienced. Domestic violence, specifically intentional physical or non- physical harm, or both, perpetuated by an intimate partner against a woman is pervasive and often an unrecognized cause of chronic physical and mental health problems. A variety of psychological and mental health symptoms such as anxiety disorders and panic attacks, depression, a sense of helplessness and declining coping skills, self blame, as well as lowered self esteem may further accompany these physical impairments. These results show that students are more likely to experience psychological abuse than physical abuse and the negative impact from the non-physical abuse is consistent with previous research findings. This research is limited to African-American college students. Further research will include an examination of differences by age, length of relationship and gender. Because the majority of the sample consisted of Alabama students interstate differences were not examined nor were urban-rural differences explored.

Table 2. Responses to Violence

Physical and Non-Physical Violence	Response		
	Broke-Off Relationship	Continued Relationship	Never Happened
When my partner hit, kicked, or slapped me and said he was sorry and wouldn't d it again, I	(9.8)	(7.0)	(70.7)
When my partner insulted, shamed or belittled me, I	(7.0)	(14.9)	(66.0)
(N=189)	*N=21*	*N=15*	*N=152*

Table. 3 Responses to Violence

Responses to Violence	Percentage
When my partner hit, kicked, or slapped me, I believe it was my fault.	
Yes	3.7
No	11.2
Never happened	71.6
If in a relationship and my partner emotionally and/or physically hurt me, I would talk to:	
A Minister	13.0
My Friend(s)	31.2
A Close Relative	18.6
A Counselor	3.3
A Police Officer or Other Law Enforcement Agents	14.9
N = 186	

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THE CHARACTERIZATION OF THREE DIGESTIVE ENZYMES FROM THE CRAYFISH *PROCAMABARUS CLARKII*

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ABSTRACT

Crayfish consume a wide variety of organisms and use a diverse array of digestive enzymes to breakdown foods. Very little is known about the enzyme reactions involved in crayfish digestion yet these reactions are crucial to absorption and assimilation. The activities of the digestive enzymes α-amylase, trypsin, and a nonspecific esterase in a crude homogenate of hepatopancreas were described in relation to changes in substrate concentration, pH, and temperature. The hepatopancreas was removed from three crayfish, homogenized in an enzyme buffer, and crudely purified using centrifugation. The activity of α-amylase increased with increasing substrate concentration and reached maximum maltose production at 15 mg/ml starch solution. The maximum velocity and apparent Michaelis constant for this reaction was approximately 328.3 units/g wet weight and 2.4 mg/ml starch, respectively. The pH optimum for α-amylase was 6.5. The activity of α-amylase increased with increasing temperature from 8 to 44 C with incremental (6 C) Q_{10} values ranging from 1.6-3.5. Trypsin activity increased with increasing substrate concentration to reach a maximum at 1.6 mM α-tosyl arginine methyl ester (TAME). The maximum velocity and apparent Michaelis constant for this reaction was approximately 342.2 units/g wet weight and 0.308 mM TAME, respectively. The pH optimum for trypsin was 8.0. The activity of trypsin increased with increasing temperature from 8 to 44 C with incremental (6 C) Q_{10} values ranging from 1.5-1.9. Nonspecific esterase activity increased with increasing substrate concentration to reach a maximum activity at 1 mM 4-nitrophenol caproate (4-NPC) solution. The maximum velocity and apparent Michaelis constant for this reaction was approximately 0.804 units/g wet weight and 0.053 mM 4-NPC, respectively. The pH optimum for the nonspecific esterase reaction was 8.5. Nonspecific esterase activity increased with increasing temperatures from 8 to 44 C with incremental (6 C) Q_{10} values ranging from 1.2-3.1. *Procambarus clarkii* has the capacity to digest carbohydrates, proteins, and lipids, typical of an omnivore. Changes in physiological or environmental conditions such as gut pH and temperature may affect the rate at which these substrates are digested in the hepatopancreas.

Crayfish, *Procamabarus clarkii*

INTRODUCTION

Crayfish are the dominant macrocrustacean in many freshwater ecosystems and are capable of feeding on a variety of plants, animals, and microorganisms, including bacteria (Brown, 1995). Native species of crayfish exist on every continent with the exception of Africa and Antarctica (Hobbs, 1988). In the United States, the crayfish *Procambarus clarkii* is the most extensively cultured crustacean (Brown, 1995).

The process of digestion in crustaceans has been described by several authors (Brown, 1995; Dall and Moriarty, 1983; Gibson and Barker, 1979; Icely and Nott, 1992; Jones et al., 1997; van Weel, 1970; Vonk, 1960). The process of chemical digestion by digestive enzymes occurs primarily in the hepatopancreas, the site for food absorption, digestive enzyme synthesis, and secretion (reviewed in van Weel, 1970; Dall and Moriarty, 1983; DeVillez and Fyler, 1985; Gibson and Barker, 1979; Icely and Nott, 1992). The analysis of α-amylase, trypsin, and nonspecific esterase in the hepatopancreas, has been shown previously to be an effective approach to understanding crustacean digestive processes (DeVillez, 1965; Brockerhoff et al., 1970; Loizzi and Peterson, 1971; DeVillez and Fyler, 1985; Biesiot, 1986; Lovett and Felder, 1990; Fang and Lee, 1992; Kamarudin et al., 1994; Jones et al., 1997). In addition, many of these previous studies have used crude digestive enzyme preparations to provide a fast and easy method to observe changes in the digestive physiology (Biesiot, 1986; Lovett and Felder, 1990; Kamarudin et al., 1994; Jones et al., 1997).

Understanding the digestive physiology of *Procambarus clarkii* may provide insight into nutrition, dietary preferences, and strategies of resource utilization that could lead to improved management strategies for aquaculture practices. Ecologically, this information may also aid in defining the niche that *Procambarus clarkii* occupies in aquatic systems. The objective of this study was to characterize crude preparations of α-amylase, trypsin and nonspecific esterase from *Procambarus clarkii.*

MATERIALS AND METHODS

Adult *Procambarus clarkii* were captured in ponds at the Louisiana State University Aquaculture Center and transported to the University of Alabama at Birmingham (UAB). They were maintained for approximately 3 months in aerated, recirculating raceways (0.6 X 2.5 m) at 27 ± 3 C with a 12 hr light-dark cycle and fed three to four times weekly (KS diet, UAB Research Foundation). Individuals were anesthetized for several minutes in ice water, blotted dry, weighed, and then dissected. The hepatopancreas was removed from each animal, weighed, and immediately placed into ice-cold general enzyme buffer (20 mM Tris-HCl with 1 mM EDTA and 10 mM calcium chloride at pH 7.4). The hepatopancreas was then homogenized using a hand-held Tissue Tearor (Biospec Products Inc.) and centrifuged (Beckman model TJ-6) at 1,500 x g for 30 min at 4 C. The supernatant was decanted into a 50 ml polypropylene tube, frozen in liquid nitrogen, and stored at –80 C until analysis. During subsequent characterizations, thawed homogenates were kept on ice for the duration of analyses.

The activity of α-amylase was measured using a kinetic endpoint assay modified from (Bernfeld, 1955). The starch substrate buffer consisted of 0.1 M sodium phosphate dibasic, 6 mM sodium chloride, and 15 mg/ml (wt/vol) starch adjusted to pH 6.9. The absorbance was determined spectrophotometrically using a Shimadzu UV-100 spectrophotometer at 540 nm

and 25 C. The optical density of the sample was computed as the average difference of the sample minus the control consisting of substrate and homogenizing buffers. The micromoles of maltose, generated by the enzymatic reaction, were derived from a standard curve generated with known amounts of maltose. The results were recorded as units per g wet weight of hepatopancreas; a unit of α-amylase activity liberates 1 μmole of maltose per minute at pH 6.9. The result was converted to units per g wet weight of hepatopancreas. All of the samples for characterization were assayed in duplicate or triplicate with single controls.

To define substrate-saturating conditions, the effects of substrate concentration were assayed. Starch solutions of 0, 1.25, 2.5, 5, 10, and 15 mg/ml were made as serial dilutions (with distilled water) from a 20 mg/ml starch (in distilled water) solution and buffered similarly (6 mM sodium chloride and 0.1 M sodium phosphate dibasic). The source of α-amylase was a 10 mg/ml (wt/vol) homogenate of hepatopancreas. To determine the optimal pH for α-amylase, assays were performed from pH 4 to 8 (in increments of 0.5 pH units). A citrate phosphate buffer was used for the pH range of 4 to 6 (0.1 M citrate, 0.1 M sodium phosphate dibasic, 0.05 M sodium chloride, and 15 mg/ml starch) and the standard buffer (0.1 M phosphate dibasic, 0.05 M sodium chloride, and 15 mg/ml starch) was used for the pH range of 6 to 8. The source of α-amylase for the pH determination was a 2.5 mg/ml (wt/vol distilled water) homogenate of hepatopancreas. The effect of assay temperature on the enzymatic activity of α-amylase was tested from 8 to 44 C (in increments of 6 C). A temperature-controlled water-circulating system (Neslab Endocal RTE-210) was used to equilibrate the test tubes to temperature prior to and during the reactions. The source of α-amylase was a 2.5 mg/ml (wt/vol) homogenate of hepatopancreas. The Q_{10} was calculated by using a modified formula to extrapolate the Q_{10} equivalent values (Prosser and DeVillez, 1991).

Trypsin was assayed using a technique described initially by (Hummel, 1959) and later modified by (Bergmeyer et al., 1974); the protocol was provided by Sigma Chemical Company (St. Louis, MO). This spectrophotometric assay uses the substrate α-tosyl-arginine-methyl-ester (TAME) dissolved in trypsin buffer (100 mM potassium phosphate dibasic and 100 mM potassium phosphate monobasic combined to a pH of 8.0) to a final concentration of 0.8 mM and then placed on ice for the duration of the assay. The absorbance was measured using a temperature-controlled Shimadzu UV-100 spectrophotometer at 247 nm and 25 C. Measurements were recorded when the change in optical density was linear (substrate concentration was saturating). Trypsin activity was measured as the average change in optical density per minute at 247 nm and then converted to units per g wet weight of hepatopancreas (1 unit = 1 micromole of TAME hydrolyzed per minute). The change in absorbance accompanying the hydrolysis of 1 μmole of TAME per milliliter of assay solution is 0.409 cm^{-1} mM^{-1} (Walsh, 1970). The result was converted to units per g wet weight of hepatopancreas. All of the samples for characterization were assayed in duplicate, and substrate-only blanks were assayed at the beginning of each analysis.

To define saturating-substrate conditions, TAME solutions of 0.016, 0.069, 0.08, 0.16, 0.27, 0.4, 0.69, 0.8, and 1.6 mM were made as dilutions (with trypsin buffer) from a 1.6 mM stock solution. The enzyme source was a 20 mg/ml (wt/vol) homogenate of hepatopancreas. To determine the optimal pH for trypsin, assays were performed from pH 4.5 to 9.5 (in 0.5

unit intervals). Trypsin buffer (100 mM potassium phosphate) was adjusted to provide buffers for the pH range of 4.5 to 8.5, and a 100 mM glycine-phosphate-sodium hydroxide buffer (pH adjusted with sodium hydroxide) was used for the range of pH 8.5 to 9.5. A 45 mg/ml (wt/vol) homogenate of hepatopancreas was used as the enzyme source for pH determinations. The effect of temperature on the enzymatic activity of trypsin was tested from 8 to 44 C (in 6 C increments). A 45 mg/ml (wt/vol) homogenate of hepatopancreas was used as the enzyme source for temperature determinations. The Q_{10} was calculated as described previously.

The activity of lipolytic enzymes (nonspecific esterases or lipases) was determined by a method modified from Bishop (1997) and Gjellesvik et al. (1992). A 100 mM stock solution of 4-nitrophenol caproate (4-NPC) was made by dissolving 4-NPC in ice-cold 100% ethanol; this solution was mixed thoroughly and aliquoted into separate borosilicate test tubes that were sealed air-tight and stored at –80 C. Aliquots of the 4-NPC stock solutions were removed from the 100 mM stock tube at the time of assay and added to ice cold buffer solution (0.5 M Tris-HCl and 0.1 M sodium chloride) at pH 8.5 (pH determined at 25 C) to give a final concentration of 0.4 mM 4-NPC. This substrate buffer solution is very sensitive to temperature, time, and pH. Substrate buffer was kept ice cold for the duration of the assay and used immediately. The absorbance was recorded from a temperature-controlled Shimadzu UV-100 spectrophotometer at 400 nm and 25 C. Measurements were recorded when the change in optical density was linear (substrate concentration was saturating). Nonspecific esterase activity was measured as the average change in optical density per minute at 400 nm and then converted to units (micromoles of 4-NPC hydrolyzed per minute) using the extinction coefficient for nitrophenol of 19,800 M^{-1} cm^{-1} at pH 8.5 and 405 nm (Gjellesvik et al., 1992). This result was converted to units per g wet weight of hepatopancreas. All of the samples for characterization were assayed in duplicate with single substrate-only controls with each assay.

To define substrate-saturating conditions, solutions of 4-NPC were made to 0, 0.025, 0.05, 0.1, 0.4, 0.6, and 1 mM. The source of nonspecific esterase was a 50 mg/ml (wt/vol) homogenate of hepatopancreas. To determine the optimal pH of nonspecific esterase activity, assays were performed from pH 6 to 11 (in increments of 0.5 pH units). The standard buffer (0.5 M Tris-HCl and 0.1 M sodium chloride) was used for the entire pH range and was adjusted with sodium hydroxide or hydrochloric acid solutions when appropriate. The source of nonspecific esterase was a 50 mg/ml (wt/vol) homogenate of hepatopancreas. The 4-NPC substrate was not pH stable and rapidly degraded at pH levels below 6 and above 11. The effect of temperature on the enzymatic activity of nonspecific esterase was tested from 8 to 44 C (in increments of 6 C). The source of nonspecific esterase was a 50 mg/ml (wt/vol) homogenate of hepatopancreas. The Q_{10} was calculated as described previously.

RESULTS

Kinetic analysis of α-amylase activity indicated that the enzyme was saturated at approximately 10 mg/ml starch and remained saturated with increasing starch concentration (Figure 1A). The maximum velocity and apparent Michaelis constant was approximately 328.3 units/g wet weight and 2.4 mg/ml starch, respectively, as determined by Lineweaver–Burk plot (Figure 2A). The enzymatic activity of α-amylase increased with increasing pH from 4 to 5.5, remained relatively constant from pH 5.5 to 6.5, and then decreased to pH 8

(Figure 1B). The enzymatic activity of α-amylase increased with increasing temperature (Figure 1C). The Q_{10} values for α-amylase ranged from 1.56 to 3.50 (Table 1).

Kinetic analysis of trypsin activity indicated that the enzyme was saturated at approximately 0.7 mM TAME and remained saturated with increasing TAME concentrations (Figure 3A). The maximum velocity and apparent Michaelis constant for this reaction were approximately 342.2 units/g wet weight and 0.308 mM TAME, respectively, as determined by Lineweaver–Burk plot (Figure 2B). The enzymatic activity of trypsin increased with increasing pH from 4.5, peaked at pH 8.0, and decreased to pH 9.5 (Figure 3B). The enzymatic activity of trypsin increased with increasing temperature (Figure 3C). The Q_{10} values for trypsin activity ranged from 1.53 to 1.88 (Table 1).

Kinetic analysis of nonspecific esterase activity indicated that the enzyme was saturated at approximately 0.8 mM 4-NPC are remained saturated with increasing substrate concentration (Figure 4A). The maximum velocity and apparent Michaelis constant for this reaction were approximately 0.804 units/g wet weight and 0.053 mM 4-NPC, respectively, as determined by Lineweaver–Burk plot (Figure 2C). The enzymatic activity of nonspecific esterase increased with increasing pH from pH 6, peaked at pH 8.5, and decreased to pH 11 (Figure 4B). The enzymatic activity of nonspecific esterase increased with increasing temperature (Figure 4C). The Q_{10} values for nonspecific esterase activity ranged from 1.21 to 3.06.

Table 1. Q_{10} Values for α-Amylase, Trypsin, and Nonspecific Esterase

Temperature °C	α-Amylase Q_{10}	Trypsin Q_{10}	Nonspecific Esterase Q_{10}
8 to 14°	3.50	1.82	2.57
14 to 20°	2.24	1.88	3.06
20 to 26°	2.10	1.60	1.40
26 to 32°	2.48	1.58	1.83
32 to 38°	1.73	1.54	1.32
38 to 44°	1.56	1.53	1.21

DISCUSSION

The digestive enzyme α-amylase exhibited substrate saturation kinetics. Similar trends in α-amylase activity were observed in larvae of the American lobster *Homarus americanus* (Biesiot, 1986). Though reports are limited in the literature for crustacea, the apparent Michaelis constant reported here (2.4-mg/ml starch) is similar to that (4.5 mg/ml) reported by Mayzaud (1985) for the copepod *Acartia clausi.*

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The optimal pH range determined for α-amylase in the crayfish *Procambarus clarkii* was pH 5.5 to 6.5. This pH range is consistent with the pH optima described previously for this species (pH 5.18 to 6.05; van Weel, 1970), the crayfish *Astacus fluviatilis* (pH 5 to 6; Kooiman, 1964) and other crustaceans (Biesiot, 1986; Blandamer and Beechey, 1964; Mayzaud, 1985; Wojtowicz and Brokerhoff, 1972).

The direct effect of temperature on the specific activity of α-amylase was similar to that described for other crustaceans. Biesiot (1986) found that α-amylase activity increased from 25 to 50 C in *Homarus americanus*. The enzymatic activity of α-amylase increased from 10 to 40 C then decreased in the crab *Carcinus maenus* (Blandamer and Beechey, 1964) and the copepod *Acartia clausi* (Mayzaud, 1985). The relatively high Q_{10} values for α-amylase in the low temperature intervals suggest that low temperatures have a greater effect on α-amylase activity than high temperatures. However, the amount of α-amylase activity present is very much greater than either trypsin or nonspecific esterase, and this reduction in specific activity probably has little physiological significance.

Trypsin also demonstrated saturation kinetics and an apparent Michaelis constant of 0.31 mM TAME. This value is consistent with the Michaelis constants of 0.33, 0.27, 0.42, and 0.39 mM TAME reported for trypsins A, B, C, and D, respectively, from *Procambarus clarkii* (Kim et al., 1994).

The optimum pH for trypsin determined in this study is 8.0. This pH is consistent with the reported optima of 8.0, 8.0, 7.5, and 7.5 for trypsins A, B, C, and D, respectively, from *Procambarus clarkii* (Kim et al., 1994), identical to that of *Orconectes virilis* (Devillez, 1965,) and slightly more acidic than the optimum pH of 8.5 reported for *Astacus astacus* (Kleine, 1967). Similar pH optima were reported for several other crustacean species, including the lobster *Homarus americanus* (Brockerhoff et al., 1970), the white shrimp *Penaeus setiferus* (Gates and Travis, 1969), the tiger prawn *Penaeus monodon* (Pei-Jung, Hsien-Ching, and Inn-Ho, 1990), and *Peneaus japonicus* (Galgani et al., 1985).

Temperature-dependent enzyme activity in the present study is comparable with the values reported for *Procambarus clarkii* (Kim et al., 1994), the crayfish *Orconectes virilis* (Devillez, 1965), and the white shrimp *Penaeus setiferus* (Gates and Travis, 1969). The low Q_{10} values for trypsin that are reported in the present study suggest that the enzymatic activity of trypsin is not substantially influenced by changes in temperature.

Similar to that of α-amylase and trypsin, nonspecific esterase activity was substrate-dependent and saturable. Though this assay has not been used (to our knowledge) for characterizations of crustacean nonspecific esterases, it has been used for larval studies with teleost fish (Bishop and Watts, 1997; Gjellesvik et al., 1992). The apparent Michaelis constant for nonspecific esterase reported in the present study is 0.053-mM 4-NPC. This is within the range reported by Gjellesvik et al. (1992) for cod (0.14 mM) and human (0.044 mM) bile salt-dependent lipase.

Nonspecific esterase exhibited an optimum pH at 8.5. This report supports the earlier study of Loizzi and Peterson (1971), who found two different Tween-60 esterases in *Procambarus clarkii*; one had an optimum at pH of 7.1 to 7.3 and the other at 8.1 to 8.4. Berner and Hammond (1969) detected two pH optima for lipolytic activity in the crayfish *Cambarus virilis*, one at pH 4.0 (a pH too acidic to be measured with 4-NPC) and another at pH 9.0 that was described as a true lipase. Kleine (1967) separated four hepatopancreatic fractions with esterase activity from the crayfish *Astacus astacus*; the pH optima of these

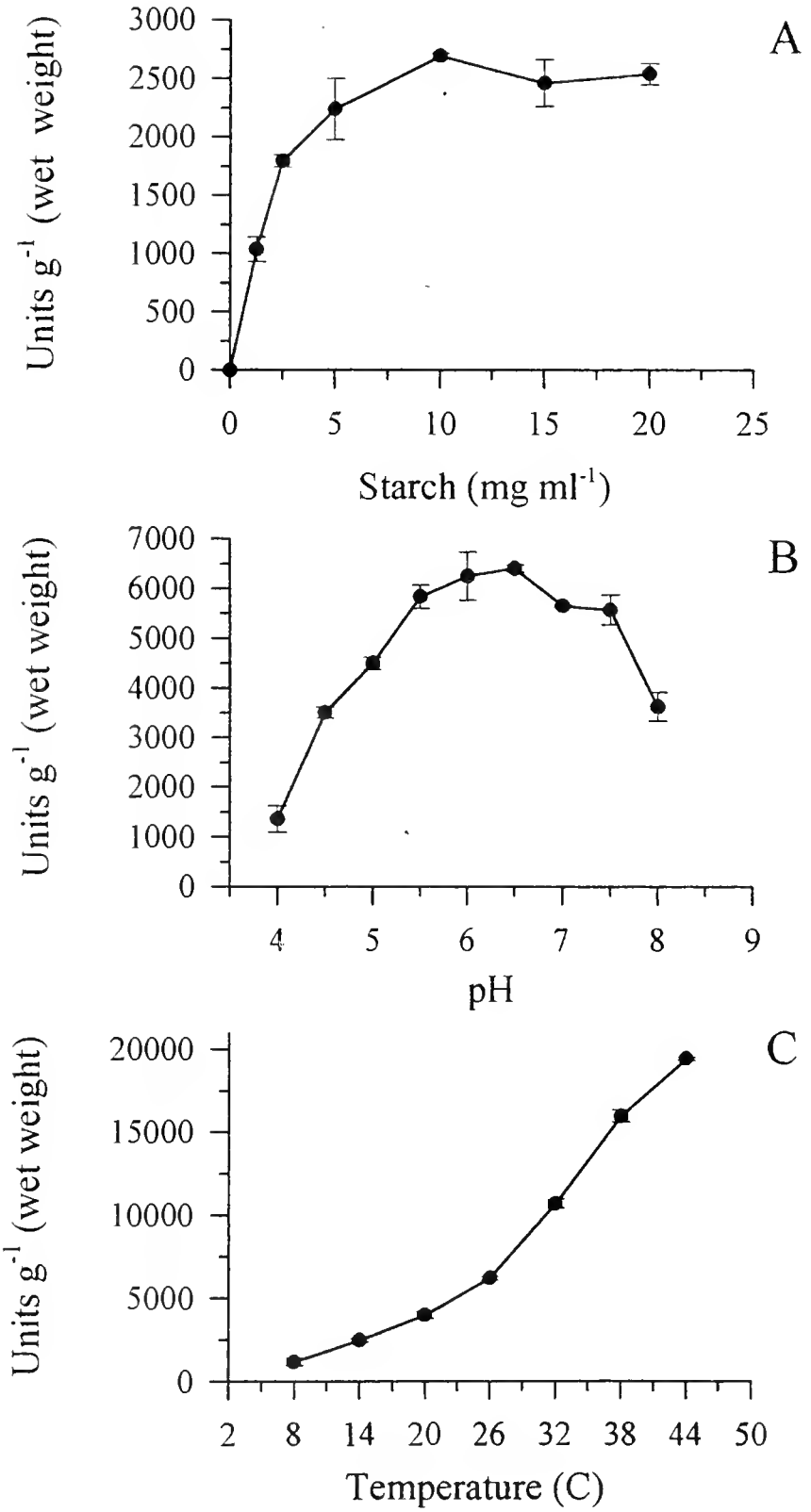

Figure 1. The effect of (A) substrate concentrations, (B) pH, and (C) assay temperature on the specific activity of α-amylase obtained from hepatopancreas extracts. Points represent the mean ± standard deviation (n = 3).

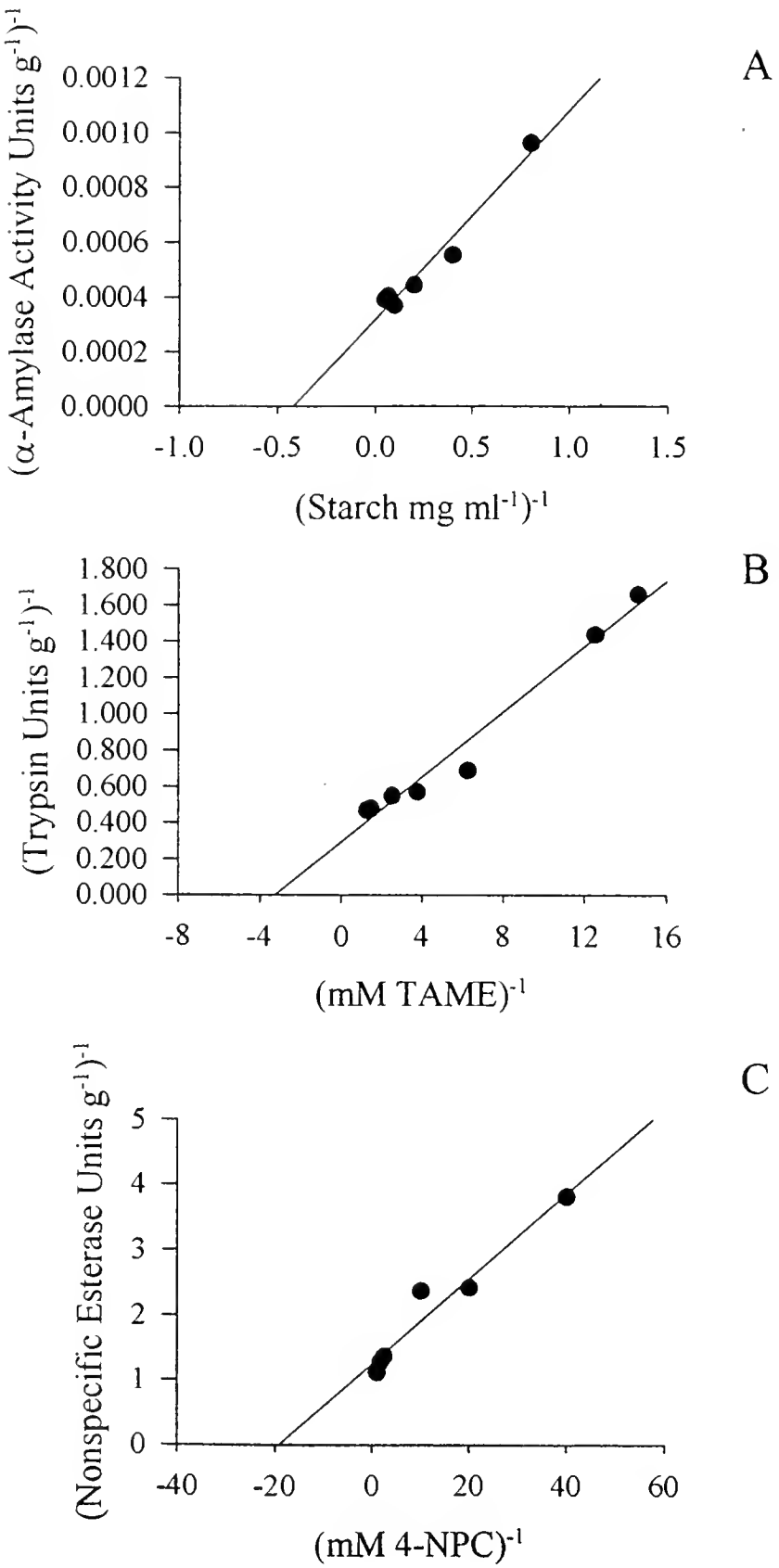

Figure 2. (A) Lineweaver Burke plots for (A) α-amylase, (B) trypsin, and (C) nonspecific esterase extracts obtained from hepatopancreas.

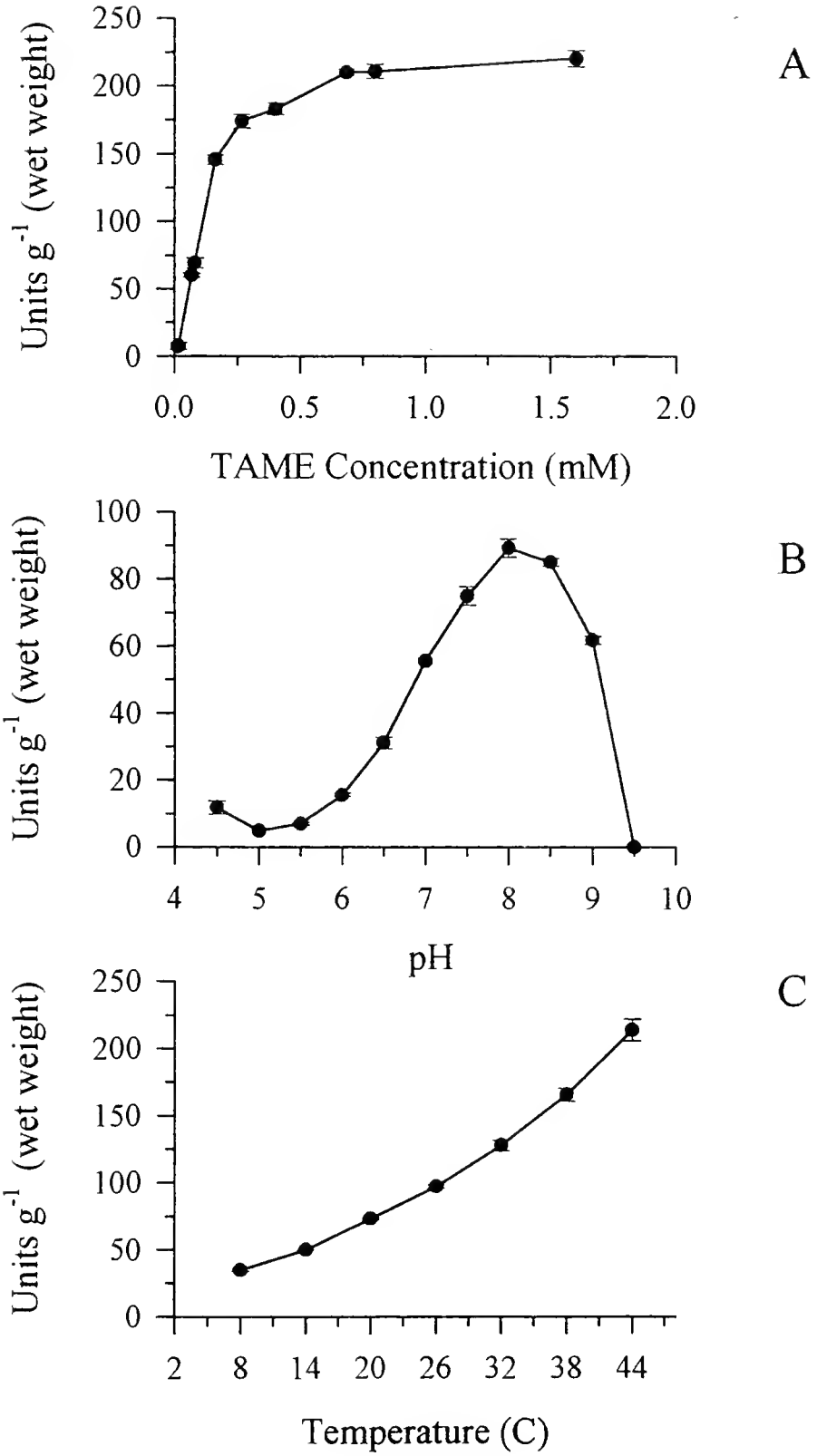

Figure 3. The effect of (A) substrate concentrations, (B) pH, and (C) assay temperature on the specific activity of trypsin obtained from hepatopancreas extracts. Points represent the mean ± standard deviation (n = 3).

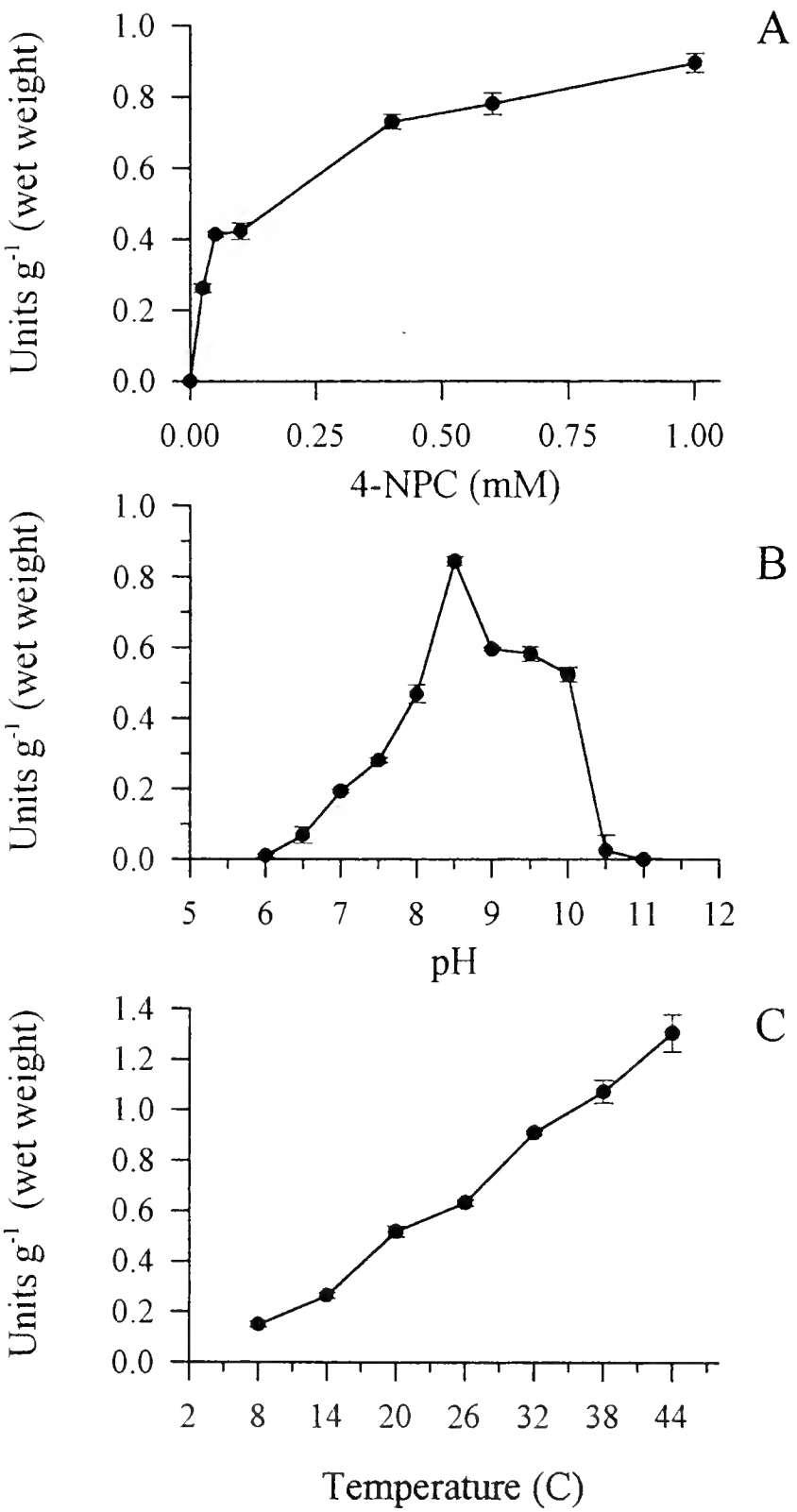

Figure 4. The effect of (A) substrate concentrations, (B) pH, and (C) assay temperature on the specific activity of nonspecific esterase obtained from hepatopancreas extracts. Points represent the mean ± standard deviation (n = 3).

enzymes were between 8 and 9. Mansour-Bek (1954) described an esterase from *Astacus sp.* that had pH optimum of 5.2 to 5.6. A true lipase has also been reported for *Homarus americanus*, with a pH optimum of 7.0 for adults (Brockerhoff et al., 1970) and 5.5 for early life history stages (Biesiot, 1986). These data suggest that crayfish, as well as other crustaceans, have several esterases that are active in the gut and hepatopancreas.

The effects of temperature on nonspecific esterase activity are consistent with those reported for lipase in *Homarus americanus* (Biesiot, 1986). The Q_{10} values for nonspecific esterase suggest that enzymatic activity is affected more by low temperature than by higher temperatures.

As suggested above, a number of factors can potentially influence the digestive capacity of the crayfish hepatopancreas, including pH and temperature. The reported pH for the crayfish gut was 4.7 to 6.6 (Brown, 1995; Gibson and Barker, 1979; van Weel, 1970), however, trypsin and nonspecific esterase have alkaline pH optima. Dall and Moriarty (1983) suggested that these enzymes may function within microenvironments (such as the diverticula of the hepatopancreas) that would allow them to function closer to the pH optima. For example, Kleine (1966) described the gastric juice of the crayfish *Astacus astacus* as being devoid of lipolytic activity but reported high lipolytic activity in an extract of hepatopancreas. Loizzi and Peterson (1971) described two Tween-60 esterases that were found at the cell-striated borders of absorptive cells and within the vacuoles of enzyme secretory and enzyme synthesizing cells in the hepatopancreas of *Procambarus clarkii.* Alternatively, Dall and Moriarty (1983) indicated that digestion may occur as fluctuations around neutral pH that would allow sufficient, but not necessarily optimum, digestion by all enzymes. These data suggest that it is possible to change the rate at which various substrates can be digested by changing the gut pH.

Procambarus clarkii maintains a capacity to digest carbohydrates, proteins, and lipids over a wide range of temperatures. At low temperatures enzyme activity is minimal. Reduced consumption rate at low temperatures (Croll, 2002) coupled with reduced enzymatic capacity, would limit the activity and growth of individuals during periods of low water temperature.

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BOOK REVIEW

GENETIC METAPHORS PROMOTE MISTAKEN NOTIONS ABOUT GENES AND FALSE FEARS AND EXPECTATIONS FOR BIOTECHNOLOGY

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The Misunderstood Gene, Michael Morange. 185 pp. plus bibliography and index, Cambridge, MA, Harvard University Press, 2001. (Transl. by Matthew Cobb)

Humans use metaphors to visualize what they cannot see or to aid their understanding of things about which they have incomplete knowledge. This is as true in science as it is in humanistic disciplines. For example, metaphorical models for the cosmos have evolved through the centuries to reflect our concepts of the structure of the universe. Now instead of envisioning stars and planets on rotating crystal spheres packed one inside each other like a set of Russian dolls after Eudoxus (4th century BCE), we imagine the expanding universe as a loaf of rising raison bread or as artist-physicist Jean-Pierre Luminet's hyperdimensional, dynamic space filled with "plunging, interpenetrating and vertiginous illogicalities" (Kemp, M. *Nature* 426: 232, 2003). Similarly, genes and their significance in specifying morphological, physiological, behavioral, and intellectual traits in plants and animals, including humans, have been represented metaphorically.

Until recently, it may not have made much difference whether one thought of an organism's genes as a book, blueprint, computer program, or deck of cards. True, as Michel Morange points out in his gem-like little book, *The Misunderstood Gene*, the predominant linguistic and computing models for genes have served population geneticists and molecular biologists for particular objectives within their disciplines, but now it is time to recognize the misunderstandings that the weaknesses of these models are engendering in the public and within a generation of scientists that has not experienced the historicity of the metaphors. The book metaphor falsely elevates the importance of genes above all other cellular components, and the computing metaphor does the same by implying that the program (DNA) commands the behavior of subservient proteins. In addition, the computing metaphor wrongly suggests that the progressive development of an organism can be understood by decoding its genes, a view that ignores the critical and complex interplay between proteins, cells, and their environment during development.

Now that we have entered the era of biotechnology when our knowledge about how cells, proteins, and genes work will soon affect individual lives and society at large in dramatic ways, how we think about gene structure and function takes on new and greater importance. The direction of genetic research, the targeting of diseases for gene therapy, the selection of traits for possible

genetic enhancement, discussion about the possible eugenic applications of biotechnology, and the public's expectations for biotechnology will all be influenced by the mental pictures that the words "gene" and "genome" conjure up. As a practicing molecular biologist and a published historian of science (Morange, M., *A History of Molecular Biology*, Harvard U. Press, 1996) in the Department of Molecular Genetics at the Ecole Normale Supérieure in Paris, Morange is well qualified to write about our misunderstanding of the gene. The Harvard University Press edition of *The Misunderstood Gene* is a translation of the original French edition published in 1998. The clarity and conciseness of the translator, Matthew Cobb, make the English edition an enjoyable read.

Morange believes that we have lost track of the reality of what genes actually do, i.e. specify proteins, the molecules upon which the material basis of all living things depend. Proteins, not genes do the real work inside cells, including the passage of genes on to successive generations via DNA replication. He believes that it is more important to understand how genes control the processes that are common to all life forms rather than dwelling on how they might specify differences between individuals. In fact, the mistaken notion that we may someday soon be able to read differences between the genomes of individual human beings and use them to predict deterministic differences in behavior or intellectual capacity makes some persons needlessly fear that biotechnology is poised to spell the end for democracy. Francis Fukuyama's recently published book, *Our Posthuman Future* (Farrar, Straus and Giroux, New York, 2002) amply illustrates Morange's point.

Proteins are extremely versatile, associating with other proteins in novel combinations to produce multisubunit proteins with diverse functions in diverse cells and tissues at diverse times during the life of an organism. Compared to our knowledge of the genome, our knowledge about the proteome (the entire array of proteins produced by a genome) is primordial. In addition, the fact that the functioning of a given protein is influenced by the population, activities, and architectural arrangements of other proteins within its microenvironment, which in turn is a product of dynamic and successive layers of larger scale environments, has been terribly underappreciated. Now, however, unexpected results from gene "knockout" experiments are beginning to force change in mistaken notions about the hegemony of the gene.

Gene knockouts involve constructing an organism that lacks a functional copy of one specific gene, giving the experimenter the opportunity to observe what effects result from the absence of the protein product of that gene. These experiments of course cannot ethically be done with human beings, but mouse gene knockouts are nearly the next best thing. The mouse genome is now sequenced, and nearly every human gene is known to have a counterpart in the mouse genome. One huge surprise for gene knockout experimenters has been the finding that knocking out a gene with a known, vital function often produces no observable defects in the organism, making it virtually impossible to precisely state what the range of function for any given gene actually is.

According to Morange, the difficulty in predicting or determining the precise function(s) of a gene, including its role in disease, arises from one or more of the following four phenomena: gene redundancy, gene compensation, networks of gene products, hierarchical structures of gene products. These phenomena force us to admit that the idea that every protein can be matched to a singular function and that all of these functions can be assembled from the ground up into an organism simply does not reflect reality. Morange could have reinforced his point even further by

mentioning the demise of the one gene - one protein concept brought about by the discoveries of alternate messenger RNA processing and RNA editing, both of which can lead to multiple gene products from a single gene.

The four phenomena identified by Morange to be forcing revisions in our concept of the gene and several examples for each of them are explained with a minimum of technical language but with detail that can only be fully appreciated by other cell or molecular biologists or by serious students in these disciplines. This makes the book less accessible to lay persons, but more valuable to those directly involved in genetic research. I suspect that Morange is betting that some of the latter who read the book are also involved in policy making or promoting the public understanding of science.

Consider gene redundancy and gene compensation which Morange states need to be distinguished from each other. "Compensation implies that the compensating gene codes for a different protein, with functions other than those of the altered protein, but that this gene will nevertheless compensate for the absence of the mutated gene...Functional redundancy fits in with the partial or total duplication of genomes that has occurred over evolution, and the resulting existence of multigene families " (p. 73-74). Results from an experiment in mice where the gene for the extracellular matrix protein, C-tenascin, has been knocked out is cited as possible evidence for the existence of functional gene redundancy. In developing amphibian embryos, the location of C-tenascin is highly site-specific and stage-specific, suggesting that it may play an important role in guiding early morphogenic cell migrations during embryogenesis. Surprisingly, mouse embryos lacking a functional C-tenascin gene developed perfectly normally. That various proteins in the extracellular matrix may have at least partially redundant functions could explain these results, or as Morange acknowledges, C-tenascin may simply not function in mice the way it does in amphibians.

The functioning of gene products within networks is another complicating factor for genetic determinists, or even for attempts to identify genes responsible for particular diseases, behaviors, or intellectual capacities. Morange uses "networks" to refer to signaling pathways that are interlaced through common protein components. He writes, "These networks are used for simple but essential tasks such as metabolic control or cell division, and for highly complex tasks such as learning and memory. The study of such networks and their mutated versions is thus a key way of discovering how genes can be implicated in complex functions despite not being specific to these functions...The signal comes from outside the cell, it interacts with the cell membrane, and it is then relayed to the inside of the cell, where it heads for the nucleus, either to activate or inhibit the expression of certain genes..." (pp. 77-78). When the protein product of a gene is a component in several signaling pathways and its roles in these are imperfectly understood, which is more often the case than not, then knocking out the gene can produce unpredicted results. For example, a multisubunit protein called NF-AT is involved in an early step in the cellular immune response. In fact, immunosuppressors given to patients receiving tissue or organ transplants act by blocking the activation of this protein. When a gene coding for one of the subunits of NF-AT was knocked out in mice, the surprising result was that the mutant mice diisplayaed lethal defects in the development of heart valves that mimic a condition causing congenital heart defects in humans. This showed that a subunit of the NF-AT protein, in addition to its role in the immune response, is also a component in a developmental signaling pathway necessary for normal heart formation. The likely existence

of hundreds or thousands of other yet unknown, interlaced signaling pathways emphasizes the absurdity of altering a single human gene in hopes of affecting only a single trait or process.

Finally, the activity any given gene product depends upon the action of multitudinous other genes comprising an ordered system of environments radiating outward with ever increasing complexity. This is what Morange calls "an organizational and structural organic hierarchy - protein machines, organelles, cells, tissues, organs, organisms, and populations" (p. 159). This hierarchy of genetic interdependency "undermines genetic determinism more than the previous arguments, so much that the very word "determinism" is no longer appropriate to describe gene function" (p. 159). Genes that regulate development like the *Hox* genes are examples of genes whose activities are nested within a complex organic hierarchy. *Hox* genes code for proteins that activate the expression of other genes, many of which code for components in intra- and intercellular signaling pathways. Such networks "provide cells with the relevant properties that enable them to interact in order to construct the organism" (p. 98) in ways that allow individuals to flourish within a population of like organisms that is in turn just one interlocking component in an ecosystem.

Modeling the molecular events at the interfaces of the various levels of hierarchical organic organization is seen by Morange as a major challenge. He uses optics as a metaphor here, speaking of the "diffraction of causal chains as they pass through these interfaces" (p. 162). What is missing from his use of this metaphor is whether he sees the incident assemblages of gene products approaching an interface as "monochromatic" or "heterchromatic." When populations of individuals are considered, I believe a "heterochromatic" metaphor would be appropriate, emphasizing the uniqueness for each individual of the intermixed genetic and environmental factors leading to the establishment of the relevant organic hierarchy. Consistent with this is a working premise of the new field of pharmacogenomics that aims someday to individually customize pharmacological prescriptions. The premise is that reading a person's genome and its state of activity in an organ or tissue will guide pharmacogenomicists in writing an optimum prescription for each individual.

Creation of the image of causal chains of protein interactions diffracting their way through hierarchical interfaces provides Morange with an ideal spot (in a future edition) to insert a chapter on proteomics, that burgeoning field of post-genomic research that aims to describe the complex webs of protein interactions from which the organism emerges. Morange gives only a single sentence to one of the most powerful techniques used in this endeavor, the yeast two-hybrid system. The absence of a discussion of proteomics is certainly forgivable though since the several new journals spawned by this discipline have appeared only within the past two years or so, well before his writing of the current book.

My list of disappointments with this book is very short, but heading it is the short shrift given to the subject of eugenics. An 11 page section at the end of the book is titled "Eugenics." Three of these are spent describing mammalian cloning as an example of a technology that was once considered infeasible but now is not. The important point that even though the genetic engineering of human traits is fraught with technical and conceptual difficulties, possible future eugenic applications of gene technologies is still worth discussing, could simply have been stated. Somatic cell and germ-line gene therapy are mentioned as two types of possible future eugenic efforts, and the latter is rightly identified as deserving the most debate. Missing from the discussion

though are other ways of categorizing eugenic practices - positive vs. negative and individual vs. population (e.g. Caplan, A.L., "What's Morally Wrong with Eugenics?" In *Controlling Our Destinies*, P. R. Sloan, ed., 2000, p. 209, University of Notre Dame Press) - and the different ethical issues arising from them. Some discourse on the difficulty in distinguishing between genetic therapy and genetic enhancement (e.g. Walters, L and J.G. Palmer, 1997. *The Ethics of Human Gene Therapy*, Oxford University Press) would also have enriched the final chapter.

The Misunderstood Gene does a very good job of exposing the myth of genetic determinism, and it convincingly points out the need for new genetic metaphors. On the last page of Chapter 1 Morange suggests "memory" as a more appropriate metaphor to describe the role of DNA and genes. A full development of the memory metaphor could be of great value to biologists ranging from population ecologists to molecular geneticists, and to the lay public as well.

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